Effects of Miscanthus grass as a fiber source in pet diets on extrusion processing and diet utilization by dogs and cats

by

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B.S., University of Sao Paulo, 2012
M.S., University of Sao Paulo, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science & Industry
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Abstract

Gastrointestinal health, digesta passage, regularity and consistency of elimination, and energy dilution of the diet can be affected by dietary fiber. Cellulose and beet pulp have been common fibers used in pet foods. Pet owners and pet food companies are in search of alternatives. Miscanthus grass is an ingredient produced from the dried canes of Miscanthus giganteus, a C4 grass grown for its high fiber content; however, it has not previously been evaluated in pet foods. Thus, the objectives were to determine the effect of Miscanthus grass on processing, nutrient utilization, hairball management, and fermentation end products. Pet foods were produced in a pilot scale extruder (E525, Extru-Tech, Sabetha, KS), dried to less than 10% moisture, then coated with chicken fat and flavor enhancer. Extrusion parameters (preconditioner and barrel water and steam addition, preconditioner discharge temperature, screw speed, die pressure, diet temperature, knife speed, specific mechanical energy, total mass flow, and wet bulk density) and kibble characteristics (kibble length, diameter, volume, density, sectional expansion ratio index, hardness, and compression energy) were evaluated for dog and cat foods produced with 10% Miscanthus grass, cellulose, or beet pulp. Miscanthus grass and cellulose dog diets required less specific mechanical energy. Additionally, these two canine diets were less dense than the beet pulp containing diet. Pet foods were fed to dogs and cats to evaluate nutrient digestibility and stool quality. Generally, dry matter, organic matter, and gross energy digestibility were lower for animals fed Miscanthus grass and cellulose diets than beet pulp diet. However, crude protein digestibility was higher for animals fed Miscanthus grass and cellulose diets compared to beet pulp diet. In both dog and cat studies, feces were softer when animals were fed the beet pulp diet. For cats, hairball management was evaluated by feeding a diet with 10% Miscanthus grass versus a non-fiber containing control diet. Most parameters evaluated

(fecal hairball count, hair masses per day, average hairball size, total hair weight) were not affected by inclusion of Miscanthus grass, but there was a trend for more hair collected on the strainer ($P = 0.0884$), less total hair per gram of dry feces, and less hair masses per gram of dry feces ($P < 0.05$). Finally, to evaluate the effects of colonic fermentation an in vitro model used canine feces as the inoculum. Five fiber sources (Miscanthus grass, cellulose, beet pulp, pea fiber, and sorghum bran) were incubated (0, 4, 8, 12h) to determine organic matter disappearance and short chain fatty acid production. Beet pulp had a higher concentration of acetate, propionate, butyrate, and total volatile fatty acids compared to all other fiber sources. Sorghum bran produced the highest amount of isobutyrate and isovalerate compared to other test fibers. Miscanthus and cellulose were very poorly fermented and generally did not differ from each other. Therefore, Miscanthus grass is an alternative fiber source that compares favorably to cellulose in dogs and cat foods without affecting processing parameters, nutrient utilization, and fermentation end products.

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Chapter 1 - Literature Review

Introduction

Pets moved from the barn into the house and are now considered members of the family by majority of pet owners. With this shift, pet owners now demand higher "nutritional standards" for the food provided to their companions. This translates into more premium and (or) wholesome ingredients (e.g., no by-products). While this may not necessarily mean that the diet is healthier and (or) better for their pets, the perception of the pet owner drives the purchase decision. Thus, there has been a compromise between the pet food producer and pet owner and it intersects at the ingredients used to produce the food. Of course, the ingredients in the formula provide nourishment. Nutrients in this sense can be split into two main categories: essential and non-essential. Essential nutrients include proteins (amino acids), fats (fatty acids), vitamins, and minerals. Surprising to some, the dispensable on nonessential nutrients include carbohydrates (starch and fibers). Even though starches and fibers are not required by the animal, they can have health benefits. For example, starch is a good source of energy, while fibers can aid in dietary energy dilution, satiation, digesta movement, and improve colonic health (Pappas et al., 1989; Sunvold et al., 1995ab). Energy dilution could be an important factor when formulating a pet food, because 56% of dogs and 60% of cats in 2017 were considered above ideal weight (Association of Pet Obesity Prevention - APOP, 2018). Obesity is considered a disease by veterinarians. It might be managed by dietary changes. Weight management could be achieved by two different approaches: increase in energy expenditure and (or) decrease in energy intake. In the first case, exercise could improve weight loss and; therefore, improve the pet's health. Alternatively, it could benefit weight loss and management by decreasing the energy content of the diet. This could be accomplished by decreasing the fat content of the diet and (or) by the

addition of fibers. Fats content could be easily reduced from the diet without any changes in the process, since fat is commonly added after the kibble is extruded and dried. Conversely, dietary fibers can be added to the food. The energy dilution effect from fiber is the result of its resistance to digestion by mammalian enzymes. Thus, fiber escapes the small intestinal digestion (and thus absorption) and enters the colon, where some of fiber's energy can be extracted by the animal indirectly from fermentation products (acetate, propionate, butyrate, lactic acid; Sunvold et al., 1995ab; Cutrignelli et al., 2009). Unlikely fat, fibers need to be added as an ingredient in the dry mix and will be processed through the extruder. Fibers are known to have negative effects on expansion of extruded products (Guy, 1994; Bouvier and Campanella, 2014) and may create sensory issues (Koppel et al., 2015). These general concepts regarding the effects of fiber addition to pet foods and companion animal nutrition will be further discussed in this literature review. To set the stage it is important to properly characterize fiber.

Fiber Characterization

Despite the common use of the terminology, fiber is a very complex and diverse group of compounds which are not easily defined or measured. Additionally, differences in raw material composition (plant variety, age at harvest, environmental conditions, and harvest date) and the process in which the fiber was produced can influence the fiber composition and concentration in the final ingredient (Fahey et al., 1990a; Cole et al., 1999). Therefore, proper characterization and detailed description of the method(s) used to determine fiber content must be reported (Fahey et al., 2018). To better understand the variation in some of the results reported in the literature, it is necessary to classify fibers. For the sake of this review, fibers will be classified by their solubility in water (soluble vs. insoluble) and their fermentability (fermentable vs. non-fermentable). Different methods are used to quantify the fiber content of ingredients and foods.

The first method developed was the crude fiber method (CFiber; Thaer, 1809 and Hennenburg and Stohmann, 1860 in 1864 in van Soest, 1964) in which the sample is digested in a strong acid and then in a base. In this procedure, all the soluble fibers are solubilized and washed out of the sample. Thus, compared to other methods, CFiber underestimates the total fiber content of the sample. However, this is the method required for the pet food label by the American Association of Feed Control Officials (AAFCO, 2018). Other methods have been developed to measure fiber in forages (van Soest, 1963; van Soest and Wine, 1967; van Soest and Wine, 1968). Like the CFiber method, several of the soluble components of the fiber are washed out of the sample and not quantified. Because these methods were not able to account for the soluble fibers, the total dietary fiber method (TDF; Prosky et al., 1985) was developed to account for the soluble fiber content in foods and food ingredients. This procedure is based on an enzymatic digestion to remove the proteins and starches from the sample. Then, the soluble fibers are precipitated with the addition of ethanol, and the residue is filtered from the solution. The fiber is estimated by weighing the dry residue after filtration. Other methods have been developed to quantify the fiber composition; however, they are not standardized and variation in the procedures and results are known to occur (Fahey et al., 2018).

Fiber Effects on the Animal's Nutrition and Health

As mentioned previously, fiber is not considered an essential nutrient for the animal. Although the consumption of fiber can be beneficial for reducing energy intake, promoting satiety, supporting gut health, and hairball management (Pappas et al., 1989; Fahey et al., 1990ab; Sunvold et al., 1995ab; Loureiro et al., 2017). The majority of pets in the US suffer from excess weight with 19.6% of dogs and 33.5% of cats reported as obese (APOP, 2018). Obesity is considered a disease which negatively impacts the long-term health of the animal. For example,

an obese animal can develop joint, cardiac, and endocrine issues, and (or) result in a chronic inflammatory state (Kealy et al., 2002; German, 2006; Laflamme et al., 2006; German et al., 2009). Thus, dietary energy restriction would be helpful. One could accomplish such energy reduction by decreasing food offered. However, this generally fails in a home setting. Alternatively, one could decrease dietary energy density by the addition of dietary fiber (Fekete et al., 2001). Because fibers are not digested by the animal, they contribute little to no energy. In addition, fiber can produce gut distention leading to the sensation of satiation. This could ameliorate begging and overcome part of the underlying behavior associated with over-feeding. The undigested fiber particles increase gut fill (distention), thus the animal feels full and reduces the sense of feeling hungry. For example, Pappas et al (1989) sham fed dogs with either a nutrient solution, inert liquid, or a water-filled balloon. Regardless of the treatment, the satiety response was proportional to the gastric distention. In a similar manner a diet rich in fiber could increase gut fill and thereby provide the stimulus necessary to activate the feedback mechanism for satiety.

The soluble undigested fibers can be fermented in the colon thereby producing short chain fatty acids (acetate, propionate, and butyrate) and branched chain fatty acids. The end products of the fermentation are used by different tissues in the body as a source of energy. For example, acetate can be used by the liver and muscle, propionate by the liver for the production of glucose (Voet et al., 2016), and butyrate is an obligate fuel for the colonocyte (Hamer et al., 2008). In addition, butyrate also has a trophic effect on the large intestine (Voet et al., 2016) which contributes to the development of the colonic tissues. The rate of fermentation and the amount of each SCFA is dependent on the fiber source (Sunvold et al., 1995ab; Biagi et al., 2008; Guevara et al., 2008). Thus, if the fiber source is concentrated in soluble and fermentable

fibers rather than insoluble and non-fermentable fibers, more SCFA will be produced (Casterline et al., 1997; Bosch et al., 2008; Cutrignelli et al., 2009).

In addition to weight issues, cats, or more specifically cat owners, are also known to suffer from hairballs. Hairballs, also known as trichobezoars, are hair masses formed in the cat's stomach due to excessive hair ingestion related to grooming (Loureiro et al., 2017). Grooming can account for up to 25% of the time that the cat is awake (Panaman, 1981). Additionally, in cases where they are group housed, grooming of other cats is part of their social behavior (Cannon, 2013). In addition to grooming habits, cats have a different fasting-state gastrointestinal contraction pattern for "housekeeping" of the upper digestive tract. The migrating spike complex is the type of gastrointestinal contractions that take place when the cat is in the fasting state (De Vos, 1993). This contraction pattern starts at the duodenum and moves until the end of the ileum, differently than the migrating motor complex in dogs (Code and Marlett, 1975). As a result, particles accumulated in the stomach (e.g. hairballs) are not moved into the duodenum like what normally happens in the dog. In addition to the hair ingestion and different gastric motility, cat's tongue anatomy may increase further the ingestion of hair after grooming (Cannon, 2013). Their tongue has a hook-like structures (filiform papillae) protruding from the top of the tongue and facing toward the back of the mouth (Weber et al., 2015). Thus, these structures make it difficult for the removal of the trapped hair other than by swallowing. Despite these peculiarities, the hairballs are usually moved from the stomach to the duodenum. However, in some cases, the hairball accumulated in the stomach is too big to pass to the duodenum and it is regurgitated (Loureiro et al., 2017) or can cause gastrointestinal blockages (Barrs et al., 1999). It is believed that the addition of fiber in the diet can decrease or eliminate this issue. For example, Davenport et al (2008) patented (patent number US 7,425,343 B2) the

use of high fiber concentrations in the diet for the purpose of improving gastric motility in an effort to pass the trichobezoars to the small intestine. Other fibers have been evaluated as well (Dann et al., 2004; Beynen et al., 2011; Loureiro et al., 2017) with variable success. Their inconsistent results may be related to different methodologies used for evaluation of animal responses and the types of fiber used. Clearly, any comparison between studies must be approached with caution.

Dietary fiber may have some drawbacks though. For example, the addition of higher levels of fiber to the diet can increase the volume of fecal excretion (Diez et al., 1998; Prola et al., 2010; Fischer et al., 2012; Sabchuk et al., 2017). Kienzle et al (1998) evaluated the digestibility of 27 dog and 24 cat foods and estimated by regression analysis the organic matter (OM) digestibility based on the dietary fiber content. Regardless of the method used to determine dietary fiber content, there was a negative linear relationship between fiber content and OM digestibility. Therefore, as fiber increases in the diet, more undigested organic material will be excreted by the animal. Despite this increased feces excreted, there are no studies relating consumer perception of fiber consumption to stool volume and (or) weight. Although it is generally assumed that smaller firmer stools are preferred by pet owners, no evidence is available to support they will reject larger firm stools. Thus, small changes in fiber and fecal volume may not be a problem. In addition to the fecal volume and (or) weight, when dietary fibers are fermented (soluble fibers), fecal dry matter decreases (Diez et al., 1998; Sabchuk et al., 2017). As fermentation increases, luminal pH decreases (Faber et al., 2011; Sabchuk et al., 2017), and the osmotic balance may shift along with an increase in postbiotic concentrations. Postbiotics are the products of the microorganism's fermentation. These would include SCFA, lactate, carbon dioxide, methane, and hydrogen gas. In response, the body may either decrease

water absorption by the colon or stimulate the secretion of water to counter balance the osmolarity and (or) increase the luminal pH. These effects are dose dependent. However, not all authors have reported an effect of the fiber sources in this manner (Flickinger et al., 2003).

Palatability is another important pet food characteristic that can be negatively impacted by dietary fiber. For example, Koppel et al (2015) reported that when given the choice between a control diet with no added fiber and test diets containing sugar cane fiber or wheat bran, that the dogs first choice and food intake always favored the control diet. Very little information is reported in the scientific literature regarding the effects of fibers on pet food acceptance; therefore, more work should be published to better understand this topic.

Fiber Effects on Extrusion Processing and Kibble Structure

Besides health, nutrition, stool quality, and palatability effects, dietary fiber inclusion brings challenges to food processing. The main effect of fiber on extruded products is a reduction in expansion. Limited data are available about the effects of fiber on extruded pet foods; therefore, this summary extrapolates results from the scientific literature for human snacks and breakfast cereals. Fiber addition in human foods has increased as consumers seek healthier alternatives. Some of these human food trends have merged with pet food as pet owners change their perspective on the relationship with their companion animals. A majority of pet foods like most breakfast cereals and some snacks are expanded extruded products. While the actual expansion happens in an instant after the product exits the extruder die, the process actually starts further upstream with hydration of the ingredient mix. The whole expansion process can be divided in five steps (Kokini et al., 1992, Moraru and Kokini, 2003): order-disorder transformation, nucleation, viscoelastic melt formation, bubble growth, and bubble collapse. The first three expansion steps somewhat overlap with each other. First is the formation of the

continuous melt (order-disorder transformation). This starts with starch hydration at the preconditioner and continues into the extruder barrel where the great majority of the starches are gelatinized and (or) melted. With the addition of water and steam in the preconditioner the starch particles start to absorb moisture, heat, and swell. As the dry mix drops into the extruder barrel the temperature increases due to the shear and friction, and the crystalline structure of the starch is lost forming a continuous molten matrix. Simultaneously in the extruder barrel, nucleation points (miniature water droplets) are created. Instabilities in the continuous starch melt created at the interface between dispersed phase fillers (material in the dry mix that is not melted or mixed with the continuous starch matrix) and equipment with the starch matrix generate embryos (i.e. nucleation points) which result in an air cell (bubble) in the final product. When these unstable areas appear, small amounts of water are trapped. In addition to the melting of the starch, a viscoelastic matrix is created. Elasticity is the force that resists the deformation caused by (steam) expansion. Although the melt elasticity is created in the extruder barrel when the starch melts, it affects the two final steps of expansion: bubble growth and collapse. Bubble growth and collapse happen after the melt exits the die. Growth of the nucleation points occurs due to the transformation of the superheated water into steam due to the difference in pressure between the inside of the extruder barrel and the ambient atmospheric conditions. As the pressure rapidly drops, the steam deforms (expands) the melt into a complex structure full of cells similar to a foam. If the forces of the steam expansion are greater than the melt elasticity, then the embryos become bubbles. If the expansion force is still greater than the elasticity, than the bubble will burst. After bursting, steam is released and elasticity forces collapse the bubbles followed by contraction of the cell walls. The collapse of bubbles continues until the temperature of the product is lower than the glass transition temperature. While these concepts are applicable to pet

foods, translation can be clouded, since the ingredient composition of a pet food is much more complex (fiber, minerals, vitamins, and fats) than an extruded breakfast cereal or snack.

One of the main ways that fiber may directly impact extrusion is by diluting the total starch content of the food. Thus, with less starch, the continuous matrix weakens since less binding material (i.e. starch) is present in the formula. Additionally, fibers have some impact in each of the expansion steps previously described. For example, fibers can compete for water with starches during hydration in the preconditioner - regardless of the type of fiber. While this may not be as important in extrusion processing, since its operation conditions to produce pet food converts ungelatinized starches into melted starches in the barrel at usual moisture levels (Rokey et al., 2010). Nucleation can be improved if the appropriate amount and type of fiber are used. For example, small amounts of finely ground insoluble fiber can improve the formation of embryos (Wang et al., 2017) and later greater expansion (Lue et al., 1991). However, soluble fibers become part of the molten matrix and thereby do not help generate nucleation sites during extrusion. Fiber addition will weaken the melt elasticity regardless of the type. Insoluble fibers are not part of the melt; thus, they break the continuity of the melt. A factor which is particularly important for bubble growth. Soluble fibers, while part of the melt, have inferior structure forming properties compared to starch. Growth of bubbles is impacted mostly by insoluble fibers. This type of fiber does not mix well with the melt and creates weak regions in the walls of the cells (Lue et al., 1991). As a result, bubbles burst prematurely. Additionally, the bubbles formed are usually elongated in the direction of the flow through the die and are shorter on the radial axis (Mendonça et al., 2000). The insoluble fiber particle aligns with the flow. This alignment can briefly strengthen the walls and force the bubbles to expand in the direction of the flow rather than radially. As a result, air cells become stretched. Finally, the bubble collapses

prematurely. Despite the brief strengthening due to the insoluble fiber particles alignment with the flow, some of the fiber particles deform and create weaker points in the cell wall where steam pressure is released (Lue et al., 1991). In addition, a thicker cell wall can accumulate more elastic energy which can contribute to a greater shrink of the bubble as product temperature decreases below the glass transition temperature. However, this hypothesis is yet to be tested.

Fiber Sources Commonly Used in Pet Foods

Because of its benefits to animal's nutrition and health, pet food companies have used a variety of fiber sources over the years to produce diets despite the challenges fibers bring to extrusion processing. They have been claimed to benefit weight management, gut health, and hairball management, among others. One of the more prominent fiber sources used in pet foods for weight and hairball management has been cellulose from the paper and pulp industry (Burrows et al., 1982; de Godoy et al., 2013a; Koppel et al., 2015). The main functionality of cellulose addition was to dilute the energy content of the diet. Cellulose is produced by chemically digesting wood chips to obtain the concentrated cellulosic material from the plant (mainly trees) cell walls (Dahl, 1884). Several researchers have experimented with cellulose in pet food applications. The consensus from the scientific literature is that cellulose is poorly fermented (Sunvold et al., 1995ab), increases fecal excretion (Wichert et al., 2002), and may decrease total tract dry matter (DM) and organic matter (OM) digestibility (Muir et al., 1996). This decrease in digestibility is somewhat connected with the poor fermentability of this ingredient. Due to the lower utilization by the colonic bacteria, more organic material (not fermented cellulose) is excreted by the animal rather than utilized as energy source by the microorganisms and the host. As a result, the DM and OM excretion is increased, which in turn decreases overall diet digestibility. In addition to weight management, cellulose addition to cat

food was shown to aid hairball excretion in the feces rather than regurgitation and (or) coughing (Beynen et al., 2011). From a processing perspective, small inclusions of finely ground cellulose can be beneficial to expansion (Kallu et al., 2017); however, these are not the usual levels evaluated in pet food related studies (Wichert et al., 2002; Prola et al., 2010). Regardless of the benefits that cellulose has provided for product function and animal health, its higher price and lack of consumer appeal has motivated pet food companies to seek alternative fiber sources. Several plant residues have been evaluated that may better fit consumer preferences such as citrus pulp, citrus pectin, fructooligosaccharides, gum Arabic, locust bean gum, oat fiber, rice bran, xanthan gum, corn fibers, wheat bran, etc. (Sunvold et al., 1995ab; de Godoy et al., 2013ab; Sa et al., 2013; Pacheco et al., 2014). Beet pulp became a standard fiber source in pet food in the 1990's. It is derived from the residue remaining after the extraction of sugar from beets. Unlike cellulose, beet pulp is known to be a moderately fermentable fiber source (Sunvold et al., 1995ab), but may still decrease DM and OM digestibility (Howard et al., 2000; Middelbos et al., 2007). It has also been reported to decrease crude protein (CP), and crude fat (CFat) total tract digestibility (Fahey et al., 1990ab; Muir et al., 1996; Sabchuk et al., 2017). As beet pulp reaches the colon and is fermented, the microbial mass increases resulting in an increased excretion of microbial protein and fat leading to an underestimate of their total tract digestibility. However, ileal digestibility was not affected by the inclusion of beet pulp (Muir et al., 1996). In this latter study, beet pulp had no negative impact on the utilization of the protein and fat content of the diet where it mattered. In addition to the increase in microbial mass, fecal dry matter of dogs fed beet pulp decreased (Fahey et al., 1990b; Fahey et al., 1992). This phenomenon is not fully understood; but it could be related to the fermentation and a shift in water absorption in the colon. One theory holds that as fermentation increases, a decrease in luminal pH (Biagi et al.,

2008; Biagi et al., 2010) could chemically irritate the epithelial layer of the large intestine and increase water secretion into the lumen (or decrease absorption) to dilute the acidity and thereby increase the luminal pH. Additionally, the increase in SCFA (Fahey et al., 1990a) concentration may shift the osmotic balance such that the pressure of the luminal content is higher than the colon. This could also result in secretion of water into the lumen and (or) decrease water absorption. Regardless of the mechanism, fecal dry matter decreased as beet pulp was added to the diet (Fahey et al., 1990a). Despite the effects on nutrient utilization, beet pulp has no effect on the hairball management in cats (Loureiro et al., 2017). With respect to expansion, as the inclusion of beet pulp in the formula increased expansion decreased (Lue et al., 1991). This may be a function, as explained previously, that fiber dilutes the starch content and thereby weakens the starch matrix resulting in less expansion. Lue et al (1991) also reported that kibbles became longer as fiber concentration increased at similar screw and knife speeds. This result indicates that, at the same feed rate, if the product does not radially expand, it will become longer, to provide an output corresponding to the feed rate.

Miscanthus Grass as an Alternative Fiber Source for Pet Foods

Despite the nutritional benefits and challenges in expansion of these standard fiber sources, all of them have a common “byproduct” origin and inferior perception by consumers. As a result, pet parents are concerned about feeding these ingredients to their companion animals. Unlike these commodity fiber sources, Miscanthus grass is a novel fiber source that could fulfill this consumer demand. Miscanthus grass is produced from the dried ground canes of Miscanthus giganteus, a C4 grass that is purposely grown for its fiber content. Originally, this grass was thought to be an ideal substrate for the production of ethanol from cellulose (Adams et al., 2018). However, this technology has yet to be economically viable. In addition to the ethanol

production, this grass has been used for construction materials, absorbents, and paper-pulping (Visser and Pignatelli, 2001). Only limited information is available in the scientific literature regarding these uses.

Interestingly, there is a constant search for new ingredients by pet food companies to meet consumer demand for novel ingredients. There is currently no research published describing the use of Miscanthus grass in animal foods and animal feeding studies. Initial nutrient analysis would suggest that Miscanthus grass may behave similar to cellulose with 85.5% vs. 97.8% measured TDF and 78.6% vs. 95.3% insoluble fiber content, respectively. So, there is a fundamental question regarding the potential utility of Miscanthus grass in animal diets and animal nutrition. Further there is a need to better understand how this fiber source might impact extrusion processing of dogs and cat foods.

The hypothesis of this work was that pet foods fortified with Miscanthus grass will behave in a similar manner during extrusion processing, and result in similar nutrient utilization, and fermentability to diets containing cellulose. The objectives were to determine the effect of Miscanthus grass on pet food processing, nutrient utilization, hairball management, and fermentation end products in comparison to other standard fiber sources.

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Chapter 2 - Effect of fiber source and particle size on chick performance and nutrient utilization

Abstract

The addition of fiber in chick feeds is known to dilute nutrients, as a result this reduces nutrient digestibility and performance. However, recent studies suggest that moderate inclusion of insoluble fibers (2 to 3%) may stimulate gizzard development, which could result in better nutrient utilization and chick growth. The previous fiber sources evaluated were subject to wide fluctuation in their nutritional and chemical composition due to variation in processing. *Miscanthus giganteus* is a C4 grass purposefully grown for its fiber content which has a consistent fiber composition compared to food process residues. The objectives of this study were to determine the effect of dietary fiber source and particle size on day-old chick performance and nutrient digestibility. Day-old chicks (8 chicks per cage, 5 cages per treatment) were fed diets containing 3% of either sepiolite (SEP), cellulose (CEL), coarse beet pulp (CBP), fine beet pulp (FBP), coarse Miscanthus grass (CMG), and fine Miscanthus grass (FMG). At the end of days 7, 14, and 21 chicks and experimental diets were weighed to compute average daily gain (ADG) and feed intake (FI). In addition, excreta from the previous 48 h of each data capture point was collected to determine nutrient digestibility. In general, chicks fed diets containing fiber consumed more feed, gained more weight and had better feed efficiency (FE) than birds fed the SEP diet. Particle size of the fiber had no effect on chick performance; however, nutrient utilization was higher ($P < 0.05$) for chicks fed coarse fiber particles compared to these fed fine fiber particles. Birds fed diets containing MG performed similar to chicks fed CEL ($P > 0.05$), but digestibility coefficients of birds fed BP diets were generally higher than chicks fed MG

diets. In conclusion, chicks performed better with fiber in their diet and MG was comparable to cellulose.

Introduction

In the past, the addition of fiber or fiber rich ingredients in poultry diets diminished animal performance (Janssen and Carré, 1985). However, recent studies contradict these original observations. For example, the addition of moderate levels of insoluble dietary fiber either had no effect or improved chick weight gain, feed intake, gain to feed ratio, digestibility, and gastrointestinal tract development (Amerah et al., 2009; Gonzalez-Alvarado et al., 2010; Hetland et al., 2004; Jimenez-Moreno et al., 2009; Jorgensen et al., 1996; Kalmendal et al., 2011; Shirzadegan and Taheri, 2017). Jimenez-Moreno et al. (2013) reported that a modest amount of fiber (approximately 2.5%) improved FCR of 1-18d old chicks without affecting DM digestibility.

While insoluble dietary fiber in moderate levels may be beneficial, the addition of soluble dietary fibers will increase digesta viscosity, slow gastric emptying, reduce feed consumption, decrease feed digestibility, and decrease ADG (Mateos et al., 2012). In addition to the type of dietary fiber (soluble vs. insoluble), the particle size may also play a role in gizzard development and improve digesta mixing with digestive secretions. Despite this, performance is usually diminished (Amerah et al., 2008; Jimenez-Moreno et al., 2010; Jorgensen et al., 1996; Mateos et al., 2012). For example, chicks fed coarse fiber particles had heavier gizzards and digestive tracts (Jimenez-Moreno et al., 2010).

Most of the fiber sources previously evaluated were a byproduct of the agricultural and food processing industries and varied in their composition depending on the plant material and the process to remove the primary valued component (Fahey et al., 1990; Montagne et al., 2003).

Miscanthus giganteus is a C4 grass grown throughout the world as an ornamental, ground cover, privacy hedge, and, in recent years, as biomass to support the growing interest in cellulosic ethanol production as an alternative fuel (Adams et al., 2018). It contains an appreciable quantity of cellulose (44 - 51%; Ververis et al., 2004; Karcher et al., 2015), an insoluble component of fiber. Different than most of the plant fibrous residues (Raninen et al., 2011), the fiber content of M. giganteus is the intended product of this crop. Therefore, it may have a more stable composition than other traditional fiber residues (Arundale et al., 2015), like beet pulp, and may be a lower cost alternative to purified insoluble fiber sources, like cellulose. Since the cellulosic ethanol technology has not yet been developed, alternative uses for MG have been explored such as: addition to construction materials, absorbents, and paper-pulping (Visser and Pignatelli, 2001). As an alternative use, it was our hypothesis that MG would be a viable alternative insoluble fiber source in poultry diets. However, there are no reports available in the literature regarding the feeding of M. giganteus to monogastric animals. Therefore, our objectives were to determine the effect of MG in day-old chick diets relative to other fiber sources on nutrient utilization and performance.

Materials and Methods

Dietary Treatments and Ingredients

The diets were formulated to meet the nutrient requirements for day-old broiler chicks according to the Nutrient Requirements for Chickens (NRC, 1994; Tables 2.1 and 2.2). Celite, an acid-washed diatomaceous earth, was added at 2% to all diets as an additional acid insoluble ash source. The control (SEP) diet contained 3% sepiolite (a complex magnesium silicate clay containing no fiber) to counter-balance the addition of test fibers (Gonzales-Alvarado et al., 2010; Jimenez-Moreno et al., 2010). All diets were fed in a meal form.

Miscanthus grass (Renew Biomass, Springfield, MO) was evaluated as coarse (100% passing a US no. 8 sieve, 2.36 mm) and fine (100% passing a no. 25 screen, 0.71 mm) ground material. The BP was from two different sources: shreds (Midwest Agri, San Rafael, CA), which were ground to pass a US no. 18 sieve (1 mm) in a hammermill, and finely ground (Fairview Mills, Seneca, KS). The cellulose (Fairview Mills, Seneca, KS) was ground to pass a no. 18 screen (1 mm). The rice, soy protein concentrate, fishmeal, ground limestone, dicalcium phosphate, salt, potassium chloride, choline chloride, DL-methionine, L-lysine hydrochloride (Lorstchers Animal Nutrition Inc., Bern, KS), soy oil (Key Feeds, Clay Center, KS), sepiolite and celite (Sigma-Aldrich Chemical Co., St. Louis, MO) were sourced prior to mixing. The base ration, excluding fiber sources, was mixed for 5 min using a horizontal double ribbon in a 454 kg double ribbon mixer, and then divided into six batches, one for each fiber source. The experimental fiber sources were added to each batch and further mixed for 5 min.

Particle size analysis (ASABE; 2008 – method S319.4) was performed in duplicate on the fiber samples to determine particle size distribution and geometric mean diameter (DGW). Thirteen sieves (sieve numbers 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, and 270) plus a pan were placed on a rotatory sieve (Testing sieve shaker model B, W.S. Tyler Inc., Mentor, OH). Each sieve and pan were cleaned with compressed air prior to analysis. One hundred grams of sample (±0.01g) was placed on the first sieve with the addition of 0.5 g of dispersing agent. After 10 minutes on the Ro-Tap, each sieve and pan were weighed. The DGW and the geometric standard deviation (SGW) were calculated using the equations below:

$$DGW = 10^{(\Sigma(W_i*0.5*\log(d_{i-1}*d_i))/\Sigma W_i)}$$

$$SGW = 0.5*DGW*((10^{S_{gw}})-(1/10^{S_{\log}}))$$

Wherein: W_i is the sample weight, d_i is the nominal sieve aperture size of the i^{th} sieve, d_{i-1} is the nominal sieve aperture size of the $i\text{-}1^{th}$ sieve, and S_{gw} is the geometric standard deviation of log-normal distribution by mass in base-ten logarithm calculated as follows:

$S_{gw} = (\Sigma(W_i*((0.5*\log(d_{i-1}*d_i))-\log(DGW))^2)/\Sigma\ Wi)^0.5$

Additionally, fiber sources were tested for acid detergent fiber (ADF) content by a commercial laboratory (Midwest Laboratories, Omaha, NE) based on the ANKOM Technology method.

Broiler Husbandry and Experimental Design

The experimental procedures for animal use were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC no. 3087). Day-old Cobb x Cobb male broiler chicks (n = 240, 52.37g average initial body weight) were obtained from a commercial hatchery. Each chick was allotted to treatment by weight. Treatments were randomly assigned to pen (5 pens per treatment, 8 chicks per pen) in a complete randomized design. Pen was considered the experimental unit. The chicks were kept on wire mesh floors, and water and feed were available throughout the duration of the experiment. Room temperature was kept constant at 29ºC±1ºC with a 24 h/d light program.

Growth Performance

Body weight and feed intake of chicks were determined by pen at 7, 14, and 21 d. The average daily gain (ADG), average daily feed intake (ADFI), and feed conversion rate (FCR) were calculated as described below. Even though pen was the experimental unit, data for performance was reported on a per bird basis. Birds that died during the experiment were weighed, as well as the feed, and results were adjusted to account for these differences accordingly.

ADG = (Final pen weight – initial pen weight)/8

ADFI = (Initial feed bucket weight – Final feed bucket weight)/(8*experimental period days)

FE = ADG/ADFI

Apparent Total Tract Nutrient Digestibility

Forty-eight hours prior to the collection of the excreta the pan underneath each cage was cleaned. After cleaning, excreta for the next 48 hours was collected on days 7, 14, and 21 for later analyses. Excreta samples were dried in a convection oven at 55ºC for 24 h until dry to touch. Dry samples were ground (Retsch Inc., Newtown PA) to pass a 1 mm screen.

Samples of feed and excreta were analyzed for dry matter (DM) by drying 1g of sample at 135ºC for 2 hours in a drying convection oven (AOAC 930.15 method). The ash content of the samples was determined by burning the samples in a muffle furnace at 600ºC overnight (AOAC 942.05). Organic matter (OM) was calculated by subtracting the ash content from the DM. Gross energy (GE) in the feed and excreta samples was determined by bomb calorimetry (Parr, Moline, IL). The apparent total tract digestibility (ATTD) of DM, OM, and GE were determined for the whole experimental period using two different methods: total excreta collection (TEC) and acid insoluble ash (AIA), as an internal marker. The acid insoluble ash content was determined as described by Van Keulen and Young (1977), with the following adaptations. Briefly, 4g of excreta and 10g of feed were burned at 600ºC overnight in a muffle furnace. On the following day, the ash residue was digested in 2 N hydrochloric acid and filtered using an ashless filter paper. Next, the filter and the samples were incinerated at 600ºC overnight. Acid insoluble ash was determined by weighing the remaining ash in the crucible. The following equations were used to calculate ATTD using TEC and AIA:

ATTD (TEC) = ((NF*FI)-(NE*EX))/ (NF*FI)

ATTD (AIA) = (1-(AIAF*NE)/(AIAE*NF))*100

wherein: NF is the percent nutrient content in the feed, FI is the feed intake in grams, NE is the percent nutrient in the excreta, EX is the amount of excreta per pen in grams, AIAF is the concentration of AIA in the feed in $g*g^{-1}$, and AIAE us the concentration of AIA in the excreta in $g*g^{-1}$. Additionally, apparent metabolizable energy (AME) was calculated as follows (Amerah et al., 2008):

AME = ((FI * diet GE) – (excreta output * excreta GE)) / FI

wherein: diet GE is the gross energy content of the diet in $kcal*kg^{-1}$, excreta output is the excreta defecated in the 48 h of TEC in a DM basis in g, and excreta GE is the gross energy of the excreta in $kcal*kg^{-1}$. Additionally, excreta output was estimated by AIA method (equation below) to determine AME.

Estimated excreta output (g) = (FI * AIAF) / AIAE

Experiment Design and Statistical Analysis

This experiment was performed as a complete randomized design, with pen as experimental unit. Performance response variables were reported per pen as an average per chick per day. Data was analyzed using statistical software (GLM procedure in SAS v. 9.4). The preplanned contrasts were SEP vs. fiber, coarse vs. fine fiber particles, CEL vs. MG, and BP vs. MG. Contrasts were considered different at alpha of 5% and trends were considered when P-value ranged from 0.05 to 0.10. Data from one pen for the CEL and CBP treatments were removed from the statistical analysis.

Results and Discussion

Particle size distribution for cellulose, BP, and MG are reported in figures 2.1, 2.2, and 2.3, respectively. Geometric mean diameter (± standard deviation) of cellulose was 96.23 ± 98.60 µm, coarse BP was 438.23 ± 366.70 µm, fine BP 276.96 ± 238.54 µm, coarse MG was 294.10 ± 253.22 µm, and fine MG 108.57 ± 66.25 µm. During evaluation for particle size clumping was observed with the first set of samples (Figure 2.4). After adding a flow agent, separation was more consistent with clumping eliminated. A closer look at the distribution of cellulose particle size showed a great variation compared to the other tested fibers (Figures 2.1, 2.2, and 2.3). This was unexpected, since cellulose is a purified ingredient with consistent chemical composition. Often, cellulose is pelleted prior to the shipment to increase the density of the product and facilitate transportation; however, if this ingredient is not properly ground prior to use, it could present a greater variation in the particle size. Despite the variation in distribution of the particles, the DGW of the cellulose used in this experiment was comparable to the DGW reported by Jimenez-Moreno et al. (2010). Both BP used in this experiment had a similar distribution of particles on the sieves. However, fine BP had two main groups of particles: first ranging from 103 to 212 µm (31.41% of the particles) and second from 420 to 594 µm (36.01% of particles), while coarse BP had 75.26% of particles concentrated between 297 and 841 µm (Figure 2.2). The DGW of both BP used in this experiment was smaller than the BP used by Jimenez-Moreno et al. (2013). When comparing with fine BP used by Jimenez-Moreno et al. (2010) in this experiment DGW was larger and the coarse BP had a smaller DGW. Coarse MG had more variation on the distribution of the particles compared to fine MG. However, while fine MG had a concentration of particles on the finer sieves and pan, both had an overlap (39.47% of particles) in the particle size distribution (Figure 2.3). This overlap in the distribution of the

particle size may account for some of the inconsistencies in the digestibility results reported in this study and will be addressed later in the discussion.

Moisture and protein content among diets was relatively similar, and slight deviation between treatments for fat composition were noted (Table 2.2). The ash level of control diet was higher due to the sepiolite exchange for fiber sources. Acid detergent fiber content of CEL and CMG were greater than other dietary treatments. Beet pulp is considered to be a moderately fermentable fiber (Fahey et al., 1990). A higher amount of fermentable fibers compared to the other tested fibers is supported by the lower content of ADF of CBP and FBP diets (2.8 and 3.1 %, respectively). During the ADF analysis soluble fibers are solubilized from the samples. However, the cellulose and lignin remain in the sample. In addition to the BP dietary treatments, the FMG diet had a lower ADF value compared to CEL and CMG diets. This may be due to the reduced particle size. Previous literature report (Alam et al., 2014) suggests that the decrease in fiber source particle size reduces the recovery of some of the insoluble components. This decrease in insoluble components of the fiber source was measured as a decrease in ADF content.

Chicks fed the SEP diet had lower ADFI compared to the other dietary treatments within each intermediate periods and the full experiment (Table 2.3). The lower ADFI impacted the ADG of these birds, resulting in lower ($P < 0.05$) FE compared to other dietary treatments. Jimenez-Moreno et al. (2010), using a similar diet, did not report higher ADFI due to the addition of fiber to diets. In their study, the ADG of chicks fed the sepiolite containing diet was lower than the diets with cellulose, oat hulls, and sugar beet pulp during 1 to 4 d and 4 to 9 d of age. Additionally, lower ADG resulted in lower FCR of birds in these same periods. When considering the later periods (9 to 15 d and 15 to 21 d) no effects due to the addition of fiber

were observed for ADFI, ADG, and FCR (Jimenez-Moreno et al., 2010). Shirzadegan and Taheri (2017) reported lower feed intake for broilers fed control diet (no added fiber) compared to the chicks fed diets containing 3 or 6% of rice bran and wood shavings.

Particle size did not affect ADFI, ADG, or FE, with the exception of higher ADG for chicks fed diets containing coarse particle fiber during 8 to 14 d of age (Table 2.3). Jimenez-Moreno et al. (2010) also evaluated coarse and fine ground fibers and did not observe any effects chick performance. Despite the differences in fiber content among the experimental treatment sources, chicks fed diets with MG performed similar to chicks fed diets containing cellulose and BP (Table 2.3). Similar results were reported by Jimenez-Moreno et al. (2013) when broilers were fed diets containing different levels of oat hulls and sugar beet pulp.

Nutrient digestibility was assessed by two different methods: total excreta collection and acid insoluble ash as an external marker (Tables 2.4 and 2.5, respectively). In general terms, both methods provided comparable results and ranked diet digestibility similarly regardless of chick age. Chicks fed fiber containing diets had higher DM digestibility and AME than chicks fed SEP diet ($P<0.05$). Organic matter and GE digestibility followed a similar pattern, with the exception of the first experimental period; wherein, a tendency for lower OM and GE digestibility for chicks fed SEP was observed ($P = 0.0912$ and $P = 0.0642$ for OM and GE digestibility respectively). Similar results were reported by Jimenez-Moreno et al. (2010), in which birds fed diets containing fiber had increased DM, OM, and nitrogen digestibility compared to chicks fed the no fiber dietary treatment. Kheravii et al. (2017) reported that the addition of lignocellulose to the diet increased CP and GE digestibility compared to no addition of fiber source. In contrast, Jorgensen et al. (1996) reported a decrease in DM, OM, and starch digestibility when broilers were fed diets with pea fiber, wheat bran, and oat bran. The inconsistent results among reports in

the literature may be explained by the different fiber inclusion levels. Jimenez-Moreno et al. (2010) included oat hulls and BP at 3%, Kheravii et al. (2017) at 1 and 2%, and Jorgensen et al. (1996) included fiber at 18.7 and 37.5%. The higher inclusion of fiber could have changed digestion dynamics and lowered nutrient digestibility.

Fiber particle size influence was also evaluated in this experiment. The inclusion of coarse particle fibers was beneficial to the chicks until they were two weeks old, as shown by the higher nutrient digestibility. Dietary addition of ingredients with coarse particle fibers was reported to stimulate gizzard development (Hetland and Svihus, 2001; Jimenez-Moreno et al., 2010; Xu et al., 2015). The gizzard controls some digestive functions like (a) particle size reduction, (b) regulation of digestive tract motility, (c) gastroduodenal reflux, (d) enrichment with hydrochloric acid, bile acid, and enzyme secretions, and (e) the management of digestion and absorption processes (Mateos et al., 2012). Therefore, the addition of coarse particles to the feed may have improved nutrient utilization by stimulating gizzard development. This was observed in this experiment by the higher digestibility coefficients of chicks fed diets containing fiber (Tables 2.4 and 2.5). Although, the addition of fiber to the diet to aid OM and GE digestibility seems to be more effective during the first and second experimental periods (earlier life stages), since no effects due to the size of the fiber particles were observed on the last experimental period. Similarly, the addition of corn with coarse or fine particles to the diet did not affect CP and GE digestibility of broilers at 24 d of age (Kheravii et al., 2017). This result suggests that at this stage in life the gastrointestinal tract is well developed and able to digest nutrients efficiently regardless of the fiber or feed particles (Amerah et al., 2008; Kheravii et al., 2017).

Chicks fed MG and CEL diets had similar DM, OM, and GE (Table 2.4) in all experimental periods. These results were expected, since both fiber sources are similar in regard to their contribution of ADF content to the diet (Table 2.2). When comparing BP with MG diets, DM digestibility was not affected. Organic matter digestibility tended to be higher for birds fed the BP diet compared to MG diets ($P > 0.05$) and GE digestibility was higher for chicks fed BP compared to chicks fed MG diets ($P < 0.05$; Table 2.4). Fiber composition is known to affect nutrient utilization by changing the digestion dynamics. For example, BP is known to contain higher quantities of pectin which could impair nutrient digestion and absorption (Mateos et al., 2012) by increasing digesta viscosity. The higher viscosity of the digesta will impair the enzyme substrate interaction and the diffusion of digested nutrients to the enterocytes (Mateos et al., 2012). Dry matter and OM digestibility of chicks fed diets containing BP was lower when compared to digestibility of diets containing oat hulls (Gonzalez-Alvaredo et al., 2010). The results presented in the current experiment were rather unexpected. The differences in BP composition may have led to these results, since the ADF content was lower than what was reported by Gonzalez-Alvaredo and others (2010).

Apparent metabolizable energy followed a similar tendency as GE digestibility (Table 2.4). Chicks fed SEP diet had lower AME than chicks fed fiber diets. The inclusion of coarse particle fibers in the diet tended to increase AME ($P = 0.0800$). Chicks fed MG diets had similar AME than chicks fed CEL diet; however, when compared to birds fed BP diets, AME was lower (Table 2.4).

Nutrient digestibility estimated by AIA followed a similar tendency as the values previously reported for TEC (Tables 2.4 and 2.5). In general, compared to no fiber addition the addition of fiber enhanced DM, OM, and GE digestibility. Chicks fed coarse fiber particles had

better nutrient utilization than chicks fed fine fiber particles, nonetheless no effects due to fiber particle size was observed on the last collection period. Broilers chick fed CEL and MG had similar nutrient digestibility. Dry matter and OM digestibilities were not affected by BP nor MG addition; however, GE digestibility tended to be higher for chicks fed BP ($P > 0.05$; Table 2.5). Similarly to AME estimated by TEC, AME determined by AIA method was ranked in a similar fashion (Table 2.5). Chicks fed fiber containing diets had a higher AME than birds fed the SEP diet for all experimental periods. Coarse fiber particle diet improved the AME compared to fine fiber particles ($P < 0.05$) for all tested periods. Broilers chick fed diets containing MG had similar AME as broilers fed CEL diet ($P > 0.05$) for all periods tested. For the first and second experimental periods, AME of chicks fed BP and MG diets was similar; however, birds fed BP diets had a higher AME than birds fed MG diets (Table 2.5).

Noy and Sklan (1997) reported that the chick is not fully developed after hatch. These authors highlighted that the villi volume in the different segments of the small intestine increase rapidly until days 4, 11, and 10 for the duodenum, jejunum, and ileum, respectively. Additionally, the trypsin, amylase, and lipase activities in the duodenum reach a peak at days 4, 7, and 10, respectively. In addition, the gizzard of chicks in the earlier life stages tested in this experiment may not have been fully developed. As reported in another publication (Mateos et al., 2012), the gizzard controls the motility, and acid and enzymatic secretions (to a lesser extent). One of the outcomes of the increase in acid secretion is the decrease in gizzard pH (Jimenez-Moreno et al., 2010). This increase in acid and enzyme secretion can favor nutrient digestibility (Gonzalez-Alvarado et al., 2010). The proposed mechanism in which the insoluble fiber particles, especially the coarse particles, will stimulate the gizzard to grind all particles before they are moved to the duodenum (Amerah et al., 2008). The grinding motion first decreases the

size of the particles in the feed, which increases the surface area for enzymatic degradation. Second, it improves the mixing of digestive secretions with the diet, which would also be beneficial to increase the digestibility of the nutrients in the lumen. Additionally, the continuous use of the gizzard will promote its hypertrophy (Jimenez-Moreno et al., 2010). Noy and Sklan (1997) also reported that the FI at this life stage may be regulated by the capacity of the chick gastrointestinal track to digest feed and absorb nutrients. Thus, the fiber addition to the diet at earlier life stages may improve gizzard development and secretion of digestive enzymes and mixing with the feed particles at a smaller particle size. The decrease in particle size and higher release of digestive secretions can improve the digestibility of nutrients. Additionally, the increased digestive capacity could lead to an increase in FI. All these factors combined could result in a greater ADG. Although in the present study organ weights and secretions were not quantified, nutrient utilization, FI, and ADG of chicks fed fiber containing diets were higher compared to the control diet. This is an indication of the underling mechanism explaining better chick performance when fiber is added to the diet. Alternatively, at latter life stages, the gizzard may be well developed and able to better control the motility and digestive secretions. As a result, the addition of dietary fiber for older chicks may have little to no benefit to chick performance and nutrient utilization. This hypothesis is supported by the lack of improvement for performance and nutrient digestibility of chicks fed coarse and fine fiber particles (Tables 2.3 and 2.4). Similar results were reported by Amerah et al. (2008), Gonzalez-Alvarado et al. (2010), and Jimenez-Moreno et al. (2011).

Excreta DM can be a useful tool to predict litter quality (Kimiaeitalab et al., 2017). Excreta of chicks fed SEP had higher DM content than excreta from birds fed diets containing fiber during the first and second excreta collection period (Table 2.6). The addition of fiber

compared to sepiolite (magnesium silicate clay) may stimulate the water secretion in the lumen by two distinct mechanisms: physical and (or) chemical irritation. The size and shape of the fiber particles may irritate the epithelial cells in the digestive tract. Lewis and Heaton (1999) reported a decrease in digesta transit time when human subjects were given plastic flakes compared to the baseline ($P < 0.05$) and a tendency of higher fecal moisture ($P = 0.08$). Tomlin and Read (1988) also reported a decrease in stool consistency of adult subjects when their diets were supplemented with 15g of plastic particles. Thus, the addition of inert particles to the diet may have a laxative effect and increase the water excretion by the birds. In addition to the physical irritation, fermentation rate of the fiber can cause chemical irritation. The faster the fiber is fermented, the higher the production of short chain fatty acids and carbon dioxide, which can acidify the luminal content (McRorie, 2015a, b) and stimulate water secretion in the colon. Due to the lower content of soluble fibers in the experimental diets and the fast digesta passage rate, the chemical irritation is unlikely to be the main cause of the decrease in excreta DM. Regardless of the differences in fiber composition, there was not an effect of fiber sources on the excreta DM (Table 2.6). Likely, mechanical irritation of the epithelial layer of the gastrointestinal tract and the ability of the fiber to retain water were the main cause of the increase in excreta water content in the fiber added diets. Coversely, Kheravii et al. (2017) reported that the addition of 1 or 2% of lignocellulose to broiler diets decreased litter moisture content. Lastly, no differences in excreta DM were reported in the last experimental period. As discussed previously, at this age, the digestive tract may be fully developed and able to reabsorb water regardless of the water holding capacity of the tested ingredients. Similarly, the addition of 3% sunflower hulls in chick diets did not affect excreta moisture content 21 d of age (Kimiaeitalab et al., 2017).

One possible shortcoming of this work was the similarity in particle size of the coarse MG and fine BP (294.10 vs. 276.96 µm for coarse MG and fine BP respectively). As reported previously, the particle size of the diet could have an impact on the performance and nutrient utilization; therefore, there may be a need to standardize the tested particle sizes before adding the fiber source to the diet. While the separation of a specific particle size in the fiber source may not be feasible for commercial scale production, for the experimental settings it may improve inferences, since it could provide a better understanding of the functionality of the fiber. Further determining the weight of digestive traits could have been a valuable addition to this study, since this is the first time to the authors knowledge that MG is been used in monogastric diets.

Additional studies to better understand the effects of fiber addition to chick diets are still necessary. Specifically, the push from consumers and government agencies to decrease or ban the use of antibiotics in sub-therapeutic doses (Kheravii et al., 2017) may provide some offsetting benefit. Fibers act as prebiotics in the large intestine and caeca and could have a benefit in selecting a healthy microbiome in the GIT of the chicks. Previously, the addition of lignocellulose at 2% contributed to a decrease in Clostridium spp. Moreover, the counts of Bifidobacterium spp., Bacteroides, Bacillus spp., and Lactobacillus spp. were not affected by 2% dietary addition of lignocellulose (Kheravii et al., 2017). Thus, more studies in this area could provide information on the type and level of fiber which might replace the use of sub-therapeutic dosages of antibiotics in the feed.

Conclusion

Overall, addition of fiber to the diets improved chick ADFI, ADG, and FE; however, there was no effect of fiber particle size and type of fiber on bird performance parameters. Nutrient utilization was improved with the addition of fiber to the diet. Acid insoluble ash as a

marker may be better than TEC, with more sensitivity to separate treatment differences. Chicks benefited from coarse fiber particle fiber in the diet in earlier stages in life, but the particle size had no benefits once the digestive tract is fully developed. The type of fiber can also have an influence on nutrient utilization. In this regard, birds fed MG diets had similar nutrient digestibility to birds fed CEL, but generally lower than chicks fed BP diets. The results indicate that MG is a safe and effective ingredient that likely has good utility as an insoluble fiber source for broilers diets.

Author Contributions to the Chapter

RAD: experiment conduction, data and sample collection, sample analysis, statistical analysis, data interpretation, and manuscript preparation.

DAS: experiment conduction, data and sample collection, and sample analysis.

CGA: experiment design, data interpretation, and manuscript revision.

RSB: data interpretation, and manuscript revision.

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Chapter 2 Figures

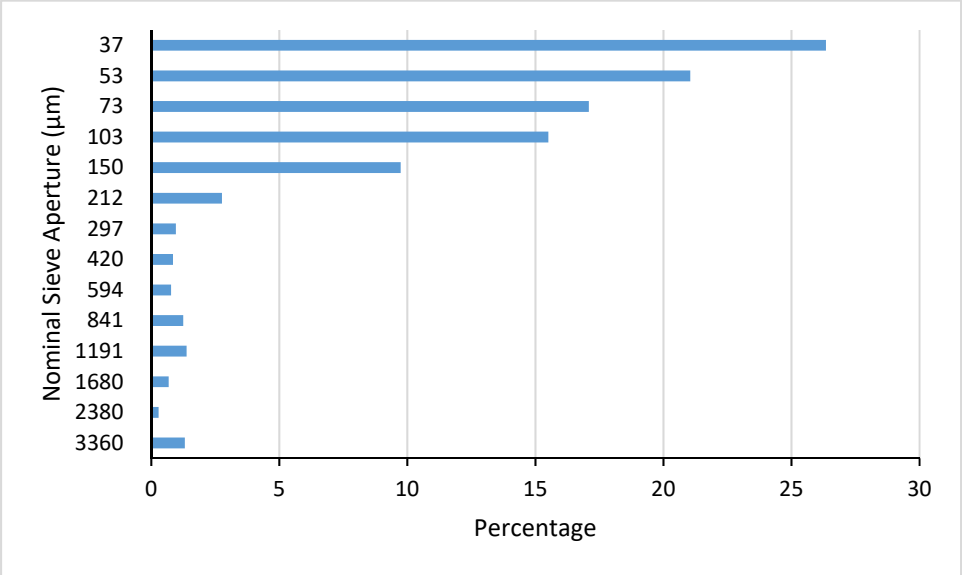

Figure 2.1 Particle size distribution of cellulose.

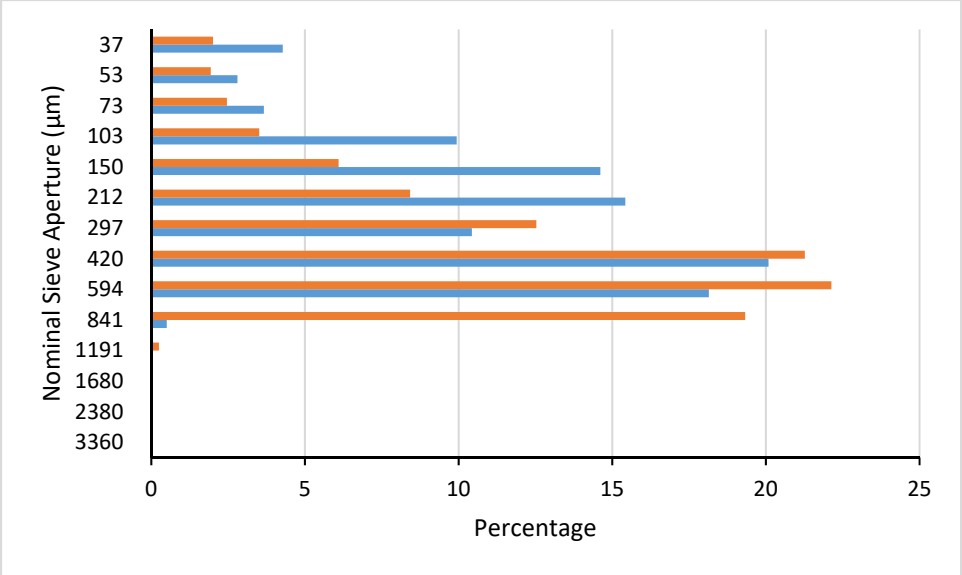

Figure 2.2 Particle size distribution of coarse (orange bars) and fine (blue bars) beet pulp.

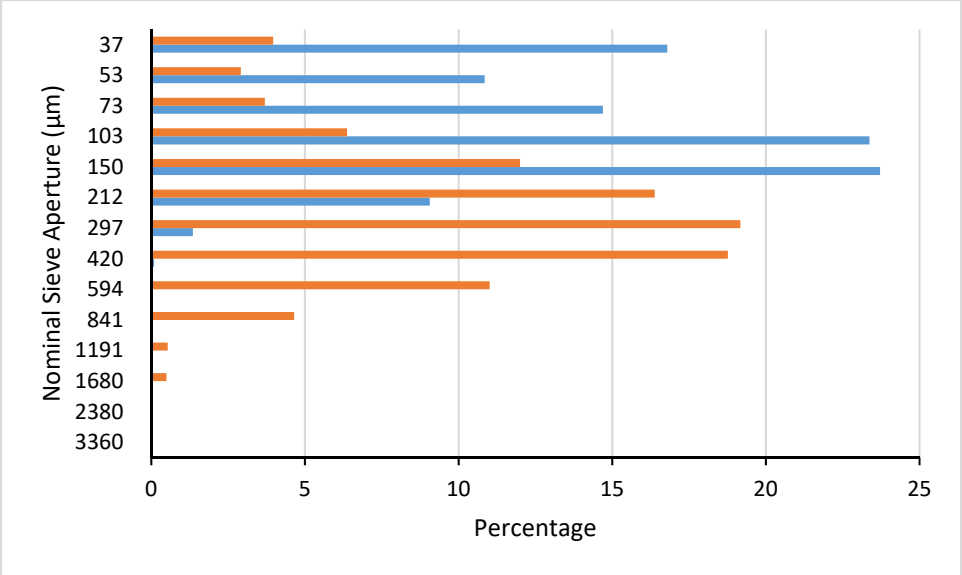

Figure 2.3 Particle size distribution of coarse (orange bars) and fine (blue bars) Miscanthus grass.

Figure 2.4 Clumps of cellulose (left) and fine Miscanthus grass (right) formed during the particle size analysis.

Chapter 2 Tables

Table 2.1 Ingredient composition of experimental diets.

Ingredient	Percent inclusion[1]
Broken rice	57.23
Soy protein concentrate, 53%CP	21.74
Fish meal, 72%CP	7.00
Soy Oil	5.00
Sepiolite/Fiber Sources	3.00
Limestone	0.85
Dicalcium phosphate	1.75
Sodium chloride	0.23
Potassium chloride	0.50
Choline chloride	0.22
L-Lys-HCl, 78%	0.02
DL-Met, 99%	0.21
Celite	2.00
Vitamin and mineral premix	0.25

[1] as fed basis.

Table 2.2 Nutrient composition of dietary treatments, % dry matter.

Diet[1]	Moisture	CP[2]	Cfat[3]	Ash	ADF[4]
SEP	10.53	24	4.57	9.23	3.4
CEL	10.44	23.5	5.71	7.6	4.3
CBP	10.32	23.7	6.09	6.9	2.8
FBP	10.08	23.8	4.98	7.32	3.1
CMG	10.17	23.8	6.28	7.19	4.1
FMG	10.24	24	5.93	7.1	3.4

[1] SEP = sepiolite, CEL = cellulose, CBP = coarse beet pulp, FBP = fine beep pulp, CMG = coarse Miscanthus grass, FMG = fine Miscanthus grass.
[2] CP = Crude protein
[3] Cfat = Crude fat
[4] ADF = Acid detergent fiber

Table 2.3 Average daily feed intake (ADFI, in g per chick per day), average daily gain (ADG, in g per chick per day), and feed efficiency (g of ADG per g of ADFI) of chicks fed diets with different fiber sources (N = 28).

Period	Days 1 - 7			Days 8 - 14			Days 15 - 21			Days 1 - 21		
Treatment[1]	ADFI	ADG	FE	ADFI	ADG	FE	ADFI	ADG	FE	ADFI	ADG	FE
SEP	15.56	14.41	0.926	30.98	22.77	0.742	42.32	26.94	0.636	29.62	21.37	0.722
CEL	19.41	15.45	0.838	32.30	26.89	0.833	46.12	32.35	0.698	32.61	24.90	0.763
CBP	16.09	16.02	0.998	35.83	30.69	0.868	49.89	36.61	0.738	33.94	27.77	0.820
FBP	16.34	15.99	0.980	34.39	30.39	0.882	51.14	36.42	0.708	33.96	27.60	0.814
CMG	18.14	16.17	0.916	35.40	31.01	0.876	49.38	34.24	0.688	34.31	27.14	0.790
FMG	15.91	15.42	0.972	33.42	28.19	0.844	50.83	36.02	0.708	33.39	26.54	0.796
RMSE[2]	2.54	0.89	0.108	2.74	1.57	0.054	4.26	4.87	0.057	2.10	1.81	0.037
Contrasts (P =)												
SEP vs. fiber	0.2099	0.0450	0.7863	0.0237	<0.0001	0.0002	0.0026	0.0025	0.0178	0.0008	<0.0001	0.0005
Coarse vs. fine	0.4066	0.3548	0.7019	0.1897	0.0425	0.7269	0.5004	0.7267	0.8576	0.6451	0.6515	1.0000
CEL vs. MG	0.1257	0.5191	0.1087	0.2064	0.0078	0.3957	0.1277	0.3445	0.9883	0.3306	0.0834	0.1793
BP vs. MG	0.4982	0.6196	0.3771	0.5867	0.2051	0.5571	0.8367	0.5454	0.3546	0.9170	0.3245	0.1753

[1] SEP = sepiolite, CEL = cellulose, CBP = coarse beet pulp, FBP = fine beep pulp, CMG = coarse Miscanthus grass, FMG = fine Miscanthus grass.

[2] RMSE = square root of the mean square error.

Table 2.4 Dry matter (DM), organic matter (OM), and gross energy (GE) total excreta collection digestibility and apparent metabolizable energy (AME) of chicks fed experimental diets with different fiber sources at different life stages (N = 28).

Period	Days 6 - 7				Days 13 - 14				Days 20 - 21			
Treatment[1]	DM	OM	GE	AME	DM	OM	GE	AME	DM	OM	GE	AME
		%		kcal/kg		%		kcal/kg		%		kcal/kg
SEP	73.81	76.46	79.17	3164.6	73.74	76.02	77.14	3083.2	75.67	78.16	79.99	3197.3
CEL	75.28	75.88	78.43	3217.9	77.86	78.60	81.00	3323.0	80.54	81.60	84.44	3464.4
CBP	79.20	80.37	83.61	3421.1	81.08	82.71	85.48	3497.5	82.67	84.10	86.77	3550.5
FBP	77.21	78.10	81.03	3356.7	78.56	79.75	81.75	3386.8	81.16	82.66	84.93	3518.1
CMG	77.43	78.02	81.20	3370.5	80.66	81.85	83.87	3481.3	80.21	81.38	84.15	3493.1
FMG	76.47	77.25	79.14	3231.1	78.00	78.96[c]	80.45	3284.4	79.89	80.95	82.80	3380.3
RMSE[2]	1.43	1.68	1.57	64.40	1.22	1.33	1.46	59.38	2.45	2.52	2.09	85.78
Contrasts (P =)												
SEP vs. fiber	0.0001	0.0912	0.0642	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0003	0.0042	0.0002	<0.0001
Coarse vs. fine	0.0360	0.0622	0.0040	0.0024	0.0001	<0.0001	<0.0001	<0.0001	0.4230	0.4312	0.1116	0.0800
CEL vs. MG	0.0599	0.0897	0.0737	0.0406	0.0539	0.0314	0.1886	0.1027	0.7407	0.7746	0.4459	0.5903
BP vs. MG	0.0710	0.0505	0.0070	0.0071	0.3906	0.1907	0.0412	0.0415	0.1112	0.0696	0.0223	0.0218

[1] SEP = sepiolite, CEL = cellulose, CBP = coarse beet pulp, FBP = fine beep pulp, CMG = coarse Miscanthus grass, FMG = fine Miscanthus grass.

[2] RMSE = square root of the mean square error.

Table 2.5 Dry matter (DM), organic matter (OM), and gross energy (GE) digestibility estimated by acid insoluble ash method of chicks fed experimental diets with different fiber sources at different life stages (N = 28).

Period	Days 6 - 7				Days 13 - 14				Days 20 - 21			
Treatment[1]	DM	OM	GE	AME	DM	OM	GE	AME	DM	OM	GE	AME
		%		kcal/kg				kcal/kg				kcal/kg
SEP	72.40	76.30	79.06	3160.2	71.19	75.62	76.76	3068.0	72.69	76.84	78.80	3149.6
CEL	72.34	74.80	77.48	3204.4	74.78	77.49	80.01	3274.7	75.64	78.64	81.94	3359.0
CBP	76.20	78.76	82.27	3356.8	78.57	81.98	84.87	3458.3	77.90	80.94	84.13	3445.2
FBP	73.25	75.31	78.61	3256.6	74.20	77.27	79.50	3293.6	75.96	79.28	81.99	3396.4
CMG	74.94	76.63	80.01	3303.4	77.51	80.49	82.66	3420.6	76.68	79.62	82.59	3424.0
FMG	73.30	75.80	77.82	3179.3	75.09	78.05	79.61	3233.4	75.58	78.36	80.45	3290.4
RMSE[2]	1.74	2.12	1.86	79.04	1.17	1.31	1.50	60.84	1.05	1.25	1.16	47.05
Contrasts (P =)												
SEP vs. fiber	0.0765	0.9711	0.8509	0.0179	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0005	<0.0001	<0.0001
Coarse vs. fine	0.0093	0.0391	0.0025	0.0067	<0.0001	<0.0001	<0.0001	<0.0001	0.0048	0.0187	0.0006	0.0005
CEL vs. MG	0.0976	0.2706	0.2050	0.4207	0.0389	0.0343	0.2193	0.1453	0.4397	0.6422	0.5563	0.9477
BP vs. MG	0.4623	0.4115	0.0880	0.0952	0.8763	0.5667	0.1422	0.1043	0.1118	0.0646	0.0090	0.0093

[1] SEP = sepiolite, CEL = cellulose, CBP = coarse beet pulp, FBP = fine beep pulp, CMG = coarse Miscanthus grass, FMG = fine Miscanthus grass.

[2] RMSE = square root of the mean square error.

Table 2.6 Excreta dry matter of chicks fed diets with different fiber sources and particle size.

Treatment[1]	Days 6 - 7	Days 13 - 14	Days 20 - 21
		%	
SEP	61.56	51.14	39.39
CEL	55.36	43.57	41.48
CBP	48.90	37.62	33.31
FBP	53.86	43.20	41.51
CMG	54.24	39.82	43.39
FMG	53.72	41.25	38.86
RMSE[2]	7.65	6.48	7.77
Contrasts (P =)			
SEP vs. fiber	0.0381	0.0047	0.9334
Coarse vs. fine	0.5360	0.2529	0.6132
CEL vs. MG	0.7621	0.4264	0.9397
BP vs. MG	0.4692	0.9676	0.3106

[1] SEP = sepiolite, CEL = cellulose, CBP = coarse beet pulp, FBP = fine beep pulp, CMG = coarse Miscanthus grass, FMG = fine Miscanthus grass.

[2] RMSE = square root of the mean square error.

Chapter 3 - The effects of fiber source on extrusion parameters and kibble structure of dry dog foods

Abstract

Traditionally, cellulose (CE) and beet pulp (BP) have been used in pet foods to assist weight management and gut health. Miscanthus grass (MG) is a novel fiber that might have similar functionality to cellulose. The objective was to determine the effects of MG, relative to CE, and BP on extrusion processing of dog foods. Diets were made with 10% of each MG, CE, or BP and 90% basal ration. Three batches of each treatment were mixed separately prior to production in a single screw extruder (E525, Extru-Tech, Sabetha, KS). During production, processing condition was recorded every 20 min after steady state was achieved. At each time point, 10 kibbles were randomly sampled for diameter and length out of the extruder and exiting the dryer. From the kibble and die diameters, sectional expansion ratio index (SEI) was calculated. Extruder parameters were similar among treatments regarding water and steam addition in the preconditioner and extruder, with the exception of specific mechanical energy (SME), wherein BP diet had higher SME than MG and CE (111.8 vs. 108.9 and 108.7 $W*h*kg^{-1}$, respectively). However, diets with BP had larger ($P<0.05$) diameter and SEI than MG and CE. Diets with BP tended ($P>0.10$) to have greater volume than MG and greater density than CE. These results indicate that MG behaved similar to CE during extrusion of dog diets. In conclusion, the extrusion parameters were not affected by fiber source, and differences in kibble characteristics can be corrected with modest process adjustments.

Introduction

Fibrous ingredients are categorized as disperse phase fillers (Guy, 1994). Fiber nutritional benefits have been explored in previous work (de Godoy et al., 2013; Sunvold et al., 1995a). While fibers can have nutritional benefits for pets, their two main effects on extrusion are to decrease expansion, and as nucleating agents. Decreased expansion is the most well-known effect. Expansion occurs due to the difference in pressure between the extruder barrel and the ambient environment. The high pressure in the barrel maintains the water as a liquid; however, when the pressure drops, the water turns into steam and expands. As a result, it creates "cells" and expands structure of the kibble. When fiber is present in the formula, it acts as needles, rupturing the air cells prematurely. As a result, steam flashes off before the cell fully expands (Guy, 1994). The second effect of fiber addition, as nucleating agent, is much more dependent on the particle size and the fiber type (insoluble fiber; Alam et al., 2014). In this case, these fiber particles will aid in the creation of the air cells necessary for the expansion and structure of the product. However, most of the effects have been tested in breakfast cereal, a matrix not nearly as complex as a pet food formula (with different ingredients and different protein, fat, mineral, starch, and moisture contents). As such, little is known about the effects of different fiber sources in dry extruded dog foods on the resulting food process.

Historically, CE, a by-product of the paper pulping industry, has been the insoluble dietary fiber standard in pet food; however, this fiber source is costly. Alternatively, other co-products of the agriculture industry have been used by the pet food companies. Beet pulp is a co-product from the sugar beet industry and has been used in pet diets. However, the fiber composition of these two ingredients is very different. Cellulose is insoluble and poorly fermentable (Sunvold et al., 1995b) and B is known to be moderately fermentable (Fahey et al.,

1990). Despite their benefits for the animals, these fiber sources have been criticized by consumers, who are concerned that agricultural co-product may be inferior for their pets.

Unlike most fiber co-products, MG is a C4 grass that is purposefully grown for its fiber content which is made by grinding the dry canes of *Miscanthus giganteus*. Originally, this crop was thought to be a substrate for the production of second generation ethanol (Adams et al., 2018). Alternative uses for this material have been explored by other industries, such as construction materials, absorbents, and paper-pulping (Visser and Pignatelli, 2001). Instead, it was our hypothesis that M could be an alternative insoluble fiber source for dry extruded dog pet foods. The objective of this study was to determine the effects of different fiber sources in extrusion parameters and kibble traits of dry expanded dog foods.

Materials and Methods

Ingredients and Dietary Treatments

Diets were formulated to meet the nutrient profile for adult maintenance (AAFCO, 2015; Table 3.1) and to be isonutritious among the treatments (Table 3.2). Miscanthus grass was provided by Renew Biomass (Springfield, MO). Chicken by-product meal, brewers rice, corn, wheat, corn gluten meal, potassium chloride, salt, choline, and vitamin and trace mineral premix were pre-blended (Fairview Mills, Seneca, KS). To this base mix each of the fiber sources along with chromic oxide and titanium dioxide were blended for 5 min in a paddle mixer (140 kg) in triplicate prior to extrusion.

Fiber sources were analyzed in duplicate for their particle size distribution according to ASABE (2008; method S319.4) in duplicates. Each sieve (sieve numbers 6, 8, 12, 16, 20, 30, 40, 50, 70, 100, 140, 200, and 270) and pan were thoroughly cleaned with compressed air prior to recording empty weight. Sample (100 ± 0.01g) was placed on the first sieve with the addition of

0.5 g of dispersing agent. After 10 minutes on the Ro-Tap (Testing sieve shaker model B, W.S. Tyler Inc., Mentor, OH), each sieve and pan was weighed. Geometric mean diameter (DGW) and the geometric standard deviation (S_{gw}) were calculated as follows:

$$DGW=10^{((\sum(W_i*0.5*\log(d_{i-1}*d_i)))/(\sum W_i))}$$

$$S_{gw}=0.5*DGW*(10^{(S_{log})}-1/(10^{(S_{log})})$$

wherein: W_i is the sample weight, d_i is the nominal sieve aperture size of the i^{th} sieve, d_{i-1} is the nominal sieve aperture size of the $i\text{-}1^{th}$ sieve, and S_{log} is the geometric standard deviation of log-normal distribution by mass in base-ten logarithm calculated as follows:

$$S_{log}=(\sum(W_i*((0.5*\log(d_{i-1}*d_i))-\log(DGW))^2/(\sum W_i))^{0.5}$$

The bulk density ($g*mL^{-1}$) of the fiber sources was measured in triplicate. Briefly a 100 mL graduated cylinder was filled until the 100 mL mark, then, it was gently shaken to remove any bridges inside the cylinder. Next the cylinder with the fiber was weighed (Sudha et al., 2007). Bulk density was calculated as follows:

$$\text{Bulk Density}=W/100$$

wherein: W is the weight of the fiber in the cylinder. The average of the three measurements for each fiber source are reported (Table 3.3).

Extrusion

During three different days one batch of each experimental diet was extruded. The order that the diets were extruded in each day was CE – BP – MG for day 1, BP – MG – CE for day 2, and MG – CE – BP for day 3. For the processing of the diets a single screw extruder (model E525, Extru-Tech, Seneca, KS) was used with a length to diameter ratio of 13.1 to 1 and a internal barrel diameter of 133.35 mm. The first five screw segments were single flight, the sixth segment was a 1.5 flight and the last segment was a conical cut double flight (Figure 3.1).

Additionally, shear locks were inserted after the fifth and sixth screw segments. The barrel profile had a spiral groove from all segments with the exception for the feeding zone. The die plate had three inserts with a single hole of 5.5 mm diameter. Feed rate and screw speed for the preconditioner and extruder were fixed at 285 $kg*h^{-1}$, 185 rpm, and 425 rpm, respectively, for all the diets to serve as a baseline for all treatments. Processing parameters (water and steam injection in the preconditioner, temperature out of the preconditioner, specific mechanical energy – SME, bulk density) were recorded every 20 min after steady state was attained.

$$SME=((\tau-\tau_0)/100*N/N_r *P_r)/m$$

wherein: SME is the specific mechanical energy in $kJ*kg^{-1}$, τ is the motor torque when in operation in N*m, τ_0 is the no load motor torque when in N*m, N is the motor speed in rpm, N_r is the rated motor speed in rpm, P_r is the motor power in W, m is the produced mass in kg. Water and steam injection in the preconditioner and extruder and knife speed were adjusted as an attempt to achieve similar out of the extruder bulk density (approximately 330 $g*L^{-1}$) among the dietary treatments.

Kibble Macrostructure

For each of the time points the extrusion parameters were collected, 10 kibbles were collected out of the extruder and measured for length once and diameter twice using a caliper for calculation of piece volume, density, and sectional expansion ratio index (SEI) as follows:

$$V=(\pi*h*D_k\text{^}2)/4$$

$$d=m_k/V$$

$$SEI=(D_k\text{^}2)/(D_d\text{^}2\ )$$

wherein: V is the volume in mL, h is the kibble length, D_k is the average of the two measurements of the kibble diameter, d is the kibble density, m is the kibble mass, SEI is the

sectional expansion ratio index, and D_d is the die hole diameter. After extrusion, each batch was dried in a convection oven set at 115.5ºC until moisture was less than 10%. Then kibbles were coated with chicken fat and flavor enhancer prior to storage in plastic bags. Prior to coating, a sample of 12 kibbles per batch was measured for length and diameter. Additionally, shrinkage was calculated as follows.

Length Shrinkage=(Dry kibble length*100)/(Wet kibble length)

Diameter Shrinkage=(Dry kibble diameter*100)/(Wet kibble diameter)

Texture Analysis

For the texture analysis a texture analyzer (model TA-XT2, Texture Technology Corp., Scarsdake, NJ) was used with a 50 kg load cell. A cylindrical probe (25 mm diameter) was used to compress 30 kibbles within each collection point for each batch. Prior to analysis, 30 kibbles from each extrusion collection point were moisture equilibrated in a convection oven for 48 h at 45ºC followed by an overnight equilibration in a desiccator. The procedure used was an adaptation from Dogan and Kokini (2007). A pre-test speed of $2mm*s^{-1}$, a test speed of $1mm*s^{-1}$ and a post-test speed of $10mm*s^{-1}$ were used. Strain level was set at 90%. Kibble hardness was considered to be the peak force in N of the first major kibble breakage, the energy to compress the kibbles to 90% was the computed area under the curve in N*mm for each compressed kibble not accounting for the negative values. The average of 90 kibbles (30 kibbles * 3 collection times) for hardness and compression energy was used as an experimental unit for statistical analysis.

Experimental Design and Statistical Analysis

This experiment was performed as a complete block design in which day was the blocking factor, and batch the experimental unit. Data were analyzed using statistical software

(SAS v. 9.4, SAS Institute, Inc., Cary, NC), by the mixed procedure of GLM (GLIMMIX), and day was considered a random factor. Least square means were considered different at alpha of 5% and trends were considered when P-value ranged from 0.05 to 0.10.

Results and Discussion

As expected, nutrient composition was similar among dietary treatments, with the exception of crude fiber content (Table 3.2). Principally, due to the composition of the fiber sources (Tables 3.2 and 3.3). Miscanthus grass had crude fiber, ADF, NDF, insoluble fiber, soluble fiber, and TDF contents in between values reported for B and C (Table 3.3). Acid detergent lignin content of MG was higher compared to BP and CE. This difference in lignin content is intrinsic to the raw materials and the process of manufacturing MG. Miscanthus grass is made by simply grinding the dry canes of *M. giganteus* without the purification step that CE undergoes in the production of pulp; therefore, the lignin present in the cane is not removed from the raw material and is present in the final product. Beet pulp had the highest density among all fibers tested, followed by MG, with CE having the lowest density. Bulk density of the ingredients can affect the density of the final product, and this will be discussed later when the bulk density of the diets is addressed. Similarly, fiber DGW impacts kibble expansion; therefore, fiber particle size will be discussed latter concurrently with SEI.

When formulating dog foods, the concentration of the nutrients in an ingredient is key for its inclusion in the diet. Therefore, if an ingredient has an elevated fiber content, less of that ingredient may be needed to meet the targeted dietary fiber level. In this case, cellulose and Miscanthus grass had a much higher fiber content than beet pulp. Thus, they could be added at smaller amounts to reach the dietary fiber target. To offset this fiber mass reduction, more starch may be added to the diet. Starch is the component of the formula that function to create the

structure of the kibble and hold all the elements altogether. Therefore, if more starch is added to the diet, or if is less diluted by other ingredients, kibble structure and expansion could be enhanced. Although this holds true to some extent, fibers are known to affect expansion.

The effects of fiber addition in extruded products are well known in the breakfast cereal and snack industries (Chinnaswamy and Hanna, 1991; Karkle et al., 2012a; Moraru and Kokini, 2003; Robin et al., 2012); however, very limited information is available regarding the effects of fiber on pet foods and pet food production. Despite the disparities in the ingredient and nutrient compositions between these human foods and pet foods, some of the well understood concepts from breakfast cereal and snack industries have value to the interpretation of results of processing pet foods. In this experiment, all three diets had the same basal ingredient composition with the fiber sources being added at 10%. Thus, differences among treatments are likely due to the fiber sources rather than the other ingredients in the diet.

It is important to remember that feed rate and screw speed for the preconditioner shaft and extruder barrel were kept constant for all diets, and other parameters were adjusted as needed to produce a pet food with targeted bulk density of 330 $g*L^{-1}$. Addition of water and steam in the preconditioner were similar among dietary treatments. This is a good indication that all tested fiber sources needed similar amounts of water to hydrate. The addition of water in the preconditioner is important for starch hydration and proper starch gelatinization in the extruder barrel (Karkle et al., 2012b). Interestingly, discharge temperature at the preconditioner of BP diet (80.8ºC) was lower than CE diet (85.1ºC; Table 4). This result could be related to the difference in the composition of these fibrous ingredients: while CE is a purified ingredient (mainly composed of cellulose from the plant cell wall), BP is composed of a variety of components that may have higher heat capacity than pure cellulose. Therefore, BP may retain more heat (added

through steam injection in the preconditioner) to reach a similar discharge temperature after the preconditioner as CE. Despite the lower temperature at the preconditioner discharge, temperature at the die was similar among dietary treatments (Table 3.4). Thus, the conditions in the extruder barrel were such to compensate for the initial temperature difference. The SME was higher for treatment BP than CE or MG (111.8 vs. 108.7 and 108.9 $J*kg^{-1}$, respectively). As noted, BP was higher in soluble fiber content than MG and CE. Since soluble fibers can increase the viscosity of the melt inside the barrel, this could result in an increase in SME. Similarly, the addition of wheat bran to extruded dog foods increased the SME compared to the addition of sugar cane fiber (Monti et al., 2016). In their work, wheat bran diet had a similar soluble fiber content to the BP diet tested in this experiment, versus a sugar cane fiber which was comparable to the CE diet. Thus, increasing dietary soluble fiber content, could result in an increase in SME. In the present experiment, only the fiber source differed among dietary treatments, thus the increase in SME is thought to be due to the increase in melt viscosity related to the increase in soluble fibers content. Despite the differences in SME, pressure at the die was similar among treatments. While statistically different, the values of SME for all diets were close to each other, and the magnitude in the increase in melt viscosity may not be sufficient to cause a change in the pressure at the die. There was no need to adjust the knife speed during production of these pet foods; however, this may have had an impact in some of the kibble measurements and this will be discussed later. As expected, since feed rate, water and steam addition in the preconditioner and extruder barrel were similar for all diets, total mass flow was not different among treatments. Finally, wet and dry bulk densities were higher for BP diet compared to CE and MG.

In general, wet BP kibbles were shorter in length than kibbles from MG and CE, although the wet kibble diameter was bigger for BP compared to the other two diets. Similar results were

reported for dry kibbles. This was reflected in the SEI; wherein kibbles from BP diet had a higher radial expansion than CE, and MG had the lowest radial expansion of all tested fiber sources (Table 3.5). Despite the higher SEI, BP kibbles piece mass was heavier than for MG and CE. Volume of kibbles from the MG diet was lower than BP and CE. As a result, the density of kibbles CE were lower than kibbles from BP (Table 3.5). The density of the kibbles could be related to two main components: the density of the fibrous ingredient added to the diet, and the effects that the fiber source had during expansion of the melt. The density of cellulose was much lower than beet pulp, with Miscanthus grass as an intermediate (Table 3.3). Thus, it is expected that even with a similar volume and smaller SEI, CE kibbles were less dense than BP kibbles. Shrinkage lengthwise was not affected by fiber source; however, diameter shrinkage was greater for MG kibbles compared to CE, with BP kibbles having the smallest diameter shrinkage (Table 3.5). Hardness and compression energy were higher for CE kibbles compared to BP and MG kibbles. In addition, BP kibbles needed less energy to be compressed than MG kibbles.

A better understanding of the bubble development process is needed to determine the extent of the influence of different fibers in expansion. While expansion happens in a fraction of a second, several models have been tested to explain the phenomenon during extrusion (Alvarez-Martinez, 1988; Della Valle et al., 1997). In simplified terms, expansion occurs because of the difference in pressure between the extruder barrel and the ambient condition, which permits the superheated steam to expand the molten starch matrix. According to Kokini et al. (1992), expansion can be divided in five main events: order-disorder transformation, nucleation, viscoelastic melt formation, bubble growth, and bubble collapse. In the first stage the starch is transformed in a continuous molten matrix. Starch gelatinization is key for the matrix creation. The starch granules start to absorb water in the preconditioner and are heated by steam injection.

The type of fiber is thought to have an impact in this step, but the effect is still to be determined. The rationale is that both soluble and insoluble fibers can compete for water and decrease the available water for starch gelatinization in the preconditioner. However, this could be of little impact for pet foods, since the usual extrusion conditions for pet food manufacturing are such that ungelatinized starch is melted. In the extruder barrel, insoluble fibers can provide additional friction, as they are usually stable to the process conditions typically used during pet food manufacturing. Simultaneous to the melt formation, nucleation begins. The formation of embryos (future air cells in the final product) happens due to some instability in the starch matrix. This variability in the melt can be accomplished by entrapment of air, fillers (fiber and other dietary components that don't mix with the molten starch), and interactions between the extrudate and equipment surfaces. Fibers can aid in nucleation; however, the benefit is dependent on the concentration, particle size, and type of fiber. Smaller inclusions of fine particles of insoluble fiber can aid in the formation of embryos, while higher concentration and bigger particle sizes can disrupt the continuity of the matrix and harm later expansion steps. The formation of the viscoelastic melt also occurs together with nucleation. The elasticity property of the melt is important to expansion because it will help to create the final structure of the kibbles. Elasticity is the combination of the forces responsible for the resistance of the melt to expansion as steam expands due to the decrease in pressure. While elasticity is generated in the barrel as the starch is melted, it is more relevant to expansion once the extrudate leaves the die opening.

Once the melt leaves the die hole, bubble growth and collapse take place. The growth of the embryos occurs as the pressure drops when the melt exits the die. The superheated steam pressure is stronger than the elasticity of the melt which will resist the deformation/expansion. The collapse of bubbles occurs after the bubble bursts open due to the forces of the expanding

steam being stronger than the elasticity of the cell walls. The role of fiber in both bubble growth and collapse is also dependent on fiber concentration, particle size, and type. Soluble fibers may diminish expansion, since it can be part of the melt as it interacts with starch. Conversely, insoluble fibers are not part of the melt and line up in the direction of flow when the cells are expanding. Thus, expansion diminishes due to weakening of the cell wall and premature cell rupture and bubble collapse, thus compromising final expansion.

With respect to these five steps, both Miscanthus grass and cellulose have a high concentration of insoluble fiber, and Miscanthus grass had a larger DGW than cellulose (Table 3.3). Therefore, although both fiber sources were added at the same concentration, the larger Miscanthus grass fiber particles may have weakened the starch melt by disrupting the continuous matrix to a greater extent than cellulose particles. Thus, cells in MG kibbles ruptured sooner than cell in CE kibbles, which accounted for the smaller SEI. This is known in the breakfast cereals and snacks scientific literature (Alam et al., 2014; Kallu et al., 2017; Wang et al., 2017). For example, Wang et al. (2017) reported that the addition of 5% cherry pomace smaller than 125 μm increased the expansion of corn starch compared to a control without added fiber. However, the addition of cherry pomace with particle sizes greater than 500 μm decreased radial expansion ratio. Additionally, while both Miscanthus grass and cellulose tested in this experiment had particle size smaller than 125 μm, it is essential to remember that there are other components in pet foods that further dilute the starch content. As a result, the pet food matrix could be more sensitive to fiber particle size than an exclusive corn starch product. Conversely, beet pulp had a higher concentration of soluble fiber and lower TDF content (Table 3.3), as a result, expansion was not jeopardized to the same extent as the other two test fibers. Expansion of the kibbles was measured in two different directions, radial and longitudinal. Insoluble fiber particles likely align

with the flow of extrudate exiting the die. This alignment could prevent radial expansion by a small reinforcement of the cell walls forcing steam to stretch the cells in the direction of the flow; therefore, the direction of expansion is more longitudinal than radial. As reported on Table 5, MG and CE kibbles were longer than BP kibbles at the same knife speed, thus, longitudinal expansion was higher to these diets with high insoluble fiber content. Similarly, Mendonça et al. (2000) reported a decrease in radial expansion when corn bran was added to snacks.

In addition to resistance cell expansion and burst, elastic energy accumulated in the cell walls can shrink the kibbles before the glass transition is achieved and the melt finally solidifies. The lower fiber content (especially insoluble fiber) of beet pulp resulted in a higher expansion compared to the two other diets. Additionally, kibbles from BP were less hard and required less energy to be compressed. All these results indicate that the cell walls of BP kibbles were thinner than cell walls from CE and MG. This supports the hypothesis that soluble fibers become part of the melted starch matrix and do not impact expansion. Similarly, Parada et al. (2011) reported that inclusion of guar gum had no effects on the expansion of extruded starchy products. Thus, it is expected that they would store less elastic energy, which reflected in less diameter shrinkage compared to CE and MG. Conversely, the higher insoluble fiber content of Miscanthus grass and cellulose caused a premature cell rupture by weakening the cell walls. As a result, the kibbles were harder and needed more energy to be compressed, supporting the idea that the cells for these two diets did not fully expand and had more elastic energy stored in them, resulting in a greater diameter shrinkage. This shrink was more pronounced in kibbles from MG than the other dog foods. As noted previously, Miscanthus grass had larger particles than cellulose, and this may be the driving mechanism to increase cell wall thickness due to premature rupture and softer kibbles, since the larger particles make them more fragile compared to smaller particles.

Additionally, Wang et al. (2017) hypothesized that the finer particles were evenly distributed in the melt, and aided nucleation of bubbles and thereby did not impact as much the bubble growth and collapse. Instead, larger particles will need more starch to surround the fiber units without damaging expansion. However, as the cell expands, the walls become thinner and the spot where the large fiber particle is becomes weak and a point for premature rupture. Monti et al. (2016) reported a decrease in piece density when fine fiber particle was used in the dog food compared to the coarse particles; however, no effects on radial expansion ratio were reported. In their study, while the expansion ratio was not different, diet with fine fiber particle was less dense, which could be an indication of more expansion, since more air would be inside the kibble to decrease the weight in the same volume.

Texture analysis can provide some insights on the micro structure of the kibbles, and it is strongly related to extrusion (Alam et al., 2014; Karkle et al., 2012a; Monti et al., 2016). In this experiment, kibbles from CE were harder than kibbles from MG and BP (Table 3.5). Kibbles from CE diet also required more energy to deform, followed by MG and BP. These results were expected. The decrease in expansion is well known to be associated with an increase in hardness (Moraru and Kokini, 2003; Yanniotis, et al., 2007). While kibbles from MG had the lowest SEI compared to the other diets, kibbles from CE were harder. This could be related to the particle size of the fiber sources. Because Miscanthus grass particles were bigger, they may have created weaker points in the micro structure of the kibbles, resulting in softer kibbles compared to CE diet. Monti et al. (2016) did not report any effect on hardness due to the addition of coarse vs. fine fiber particles. Similarly, Alvarenga et al. (2018) did not report an increase in hardness of dog foods when sorghum mill-feed was added compared to the control and whole sorghum diets; however, these authors reported a bigger standard error for the diet containing sorghum mill-

feed. Conversely, the addition of pectin to extruded corn starch decreased hardness compared to the addition of wheat fiber (Yanniotis et al., 2007). Similar results were reported in our study; wherein, the addition of beet pulp to the diet decreased hardness compared to the addition of Miscanthus grass and cellulose (Table 3.5). Energy to compress mirrored the results from hardness; wherein, more energy was required to compress kibbles for CE than MG, and kibbles from BP required the least. Alvarenga et al. (2018) added sorghum mill-feed to dog foods and did not report an increase in the amount of energy necessary to compress the kibbles. This could be related to the decrease in total starch of the diet compared to the control diet (46.9 % vs. 35.3 %, respectively for control and mill-feed diets). Even though the addition of fiber to the diet is known to increase the hardness, the dilution of starch in this case may have damaged the micro structure of the kibbles to an extent that similar hardness and energy to compress were reported.

In summary, the addition of fiber to extruded products is known to impact extrusion. While similar amounts of water and steam were added to these diets, preconditioner discharge temperature was lower for BP diet compared to CE. Additionally, SME was slightly higher for BP compared to the other tested fibers. Bulk density in this case was not just related to the expansion of the melt, but also to the density of the fiber source; wherein, beet pulp was denser than the Miscanthus grass and cellulose. The effects of fiber addition on expansion is dependent on the fiber type, particle size, and inclusion level. Generally, soluble fibers damage less radial expansion compared to insoluble fibers. However, if the appropriate particle size and content are used, insoluble fibers could increase expansion further than no fiber supplementation. In this case the addition of Miscanthus grass and cellulose decreased SEI compared to beet pulp inclusion. As expected, with a decrease in SEI, hardness of MG and CE kibbles increased compared to kibbles from BP.

Conclusion

In conclusion, Miscanthus grass has a fiber profile more similar to cellulose than beet pulp. Thus, during extrusion of dog foods, it behaved in a similar fashion to cellulose (similar water and steam injection, preconditioner discharge temperature, SME, and wet and dry bulk densities). In addition to the extrusion parameters, kibble measurements of MG were closely related to CE (length, diameter, and weight). Therefore, Miscanthus grass could be used as a replacement of cellulose if small changes in extrusion condition were adopted. Further, this work demonstrated a relationship between type of fiber and particle size on kibble microstructure as expansion decreased when insoluble fiber of large particles was added to the diet.

Author Contributions to the Chapter

RAD: experiment conduction, data and sample collection, sample analysis, statistical analysis, data interpretation, and manuscript preparation.

CGA: experiment design, data interpretation, and manuscript revision.

HD: texture analysis.

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Chapter 3 Figures

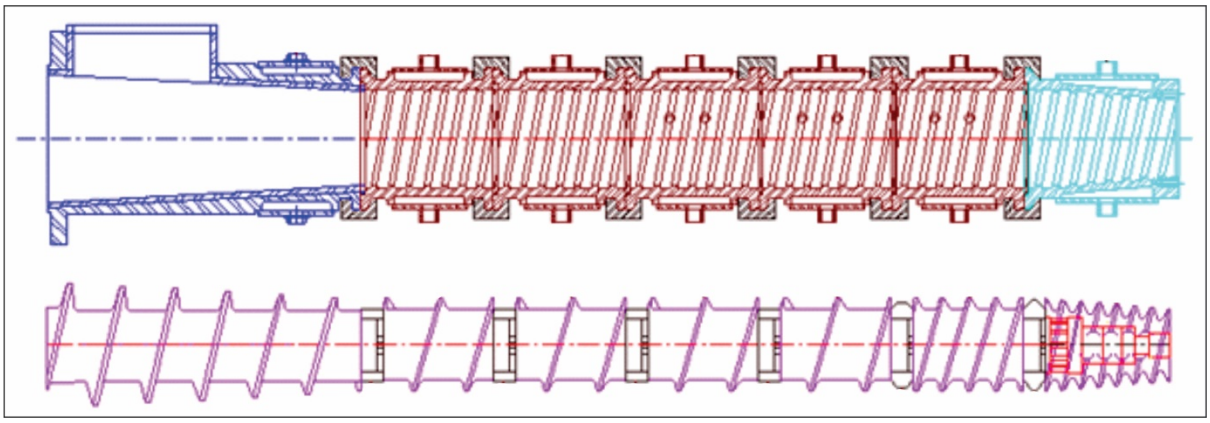

Figure 3.1 Screw and barrel profile of E525 extruder. Inlet on the left side, outlet on the right.

Chapter 3 Tables

Table 3.1 Ingredient composition of experimental diets, which were mixed and ground to pass a number 16 screen prior to extrusion.

Ingredient	%
Chicken by-product meal (low ash)	31.59
Brewers rice	18.05
Corn	18.05
Wheat	15.35
Fiber source	10.00
Corn gluten meal	5.42
Potassium chloride	0.32
Salt	0.52
Titanium dioxide	0.40
Chromic oxide	0.25
Choline chloride, 60%	0.24
Natural antioxidant	0.18
Vitamin premix[1]	0.16
Trace mineral premix[2]	0.11

[1] Vitamin E Supplement (79,887 IU*kg^{-1}), Niacin Supplement (64,736 mg*kg^{-1}), Calcium Pantothenate (12,186 mg*kg^{-1}), Vitamin A Supplement (17,162,998 IU*kg^{-1}), Thiamin Mononitrate (14,252 mg*kg^{-1}), Pyridoxine Hydrochloride (5,537 mg*kg^{-1}), Riboflavin Supplement (4,719 mg*kg^{-1}), Vitamin D3 Supplement (920,000 IU*kg^{-1}), Biotin (70 mg*kg^{-1}), Vitamin B12 Supplement (22 mg*kg^{-1}), Folic Acid (720 mg*kg^{-1}), as is basis.

[2] Zinc Sulfate (88,000 mg*kg^{-1}), Ferrous Sulfate (38,910 mg*kg^{-1}), Copper Sulfate (11,234 mg*kg^{-1}), Manganous Oxide (5,842 mg*kg^{-1}), Sodium Selenite (310 mg*kg^{-1}), Calcium Iodate (1,584 mg*kg^{-1}), as is basis.

Table 3.2 Nutrient composition of dietary treatments (dry matter basis) produced to evaluate processing parameters during extrusion and after drying.

Composition	MG [1]	CE [1]	BP [1]
Dry matter	94.30	95.39	95.19
Crude protein	34.90	32.00	33.00
Crude fat	10.10	8.30	8.71
Ash	6.86	6.58	7.55
Crude fiber	6.76	9.06	4.13

[1] MG: Miscanthus grass diet, CE: cellulose diet, BP: beet pulp diet.

Table 3.3 Fiber profile and bulk density of tested fiber sources (dry matter basis).

Composition	Miscanthus Grass	Cellulose	Beet Pulp
Dry matter, %	95.00	95.30	92.53
Crude fiber, %	47.58	76.29	20.21
Acid detergent fiber, %	56.53	84.58	26.26
Neutral detergent fiber, %	77.68	92.76	34.15
Acid detergent lignin, %	13.68	0.73	6.38
Total dietary fiber, %	90.00	102.62	62.36
Insoluble fiber, %	82.74	100.00	35.99
Soluble fiber, %	7.26	2.62	26.37
Bulk Density, $g*mL^{-1}$	0.31	0.19	0.73
DGW ± S_{gw}, µm [1]	103.46 ± 76.39	77.33 ± 44.47	193.78 ± 194.83

[1] DGW = Geometric Mean Diameter, S_{gw} = Standard Deviation

Table 3.4 Processing conditions of dog foods with different fiber sources.

Parameter	MG	CE	BP	SEM [1]	P-value
Feed rate, kg*h^{-1}	286.4	286.4	286.4	0.022	0.4444
Feed Bulk Density, g*L^{-1}	641	641	641		
Preconditioner					
Shaft speed, rpm	185	185	185		
Water, kg*h^{-1}	37.2	38.2	37.5	1.55	0.3452
Steam, kg*h^{-1}	66.3	72.1	58.4	7.67	0.4743
Temperature, ºC	83.4^{ab}	85.1^{a}	80.8^{b}	1.99	0.0807
Extruder					
Screw speed, rpm	425.00	425.00	425.00		
Water, kg*h^{-1}	0.00	0.03	0.05	0.034	0.6109
Steam, kg*h^{-1}	0.83	0.83	0.83		
Die					
Temperature, ºC	138.4	140.3	139.3	3.11	0.8276
Pressure, psi	200	200	200		
Knife speed, rpm	800	800	800		
Other					
Specific Mechanical Energy, J*kg^{-1}	108.9^{b}	108.7^{b}	111.8^{a}	1.18	0.0185
Total Mass Flow, kg*h^{-1}	337	339	336	1.37	0.4104
Diet Wet Bulk Density, g*L^{-1}	330^{a}	292^{b}	339^{a}	9.50	0.0496

^{ab} Means with unlike superscripts differ, $P < 0.05$.

[1] SEM: standard error of the mean.

Table 3.5 Kibble characteristics out of the extruder and drier, shrinkage, and macrostructure of dry dog foods with varying fiber sources.

Kibble parameter	MG	CE	BP	SEM [1]	P-Value
Out of the extruder					
Length, mm	9.29[a]	9.32[a]	8.02[b]	0.34	0.0019
Diameter, mm	11.15[b]	11.33[b]	12.13[a]	0.16	0.0046
Out of the drier					
Length, mm	8.94[a]	9.00[a]	7.46[b]	0.26	0.0030
Diameter, mm	10.21[c]	10.58[b]	11.69[a]	0.05	<0.0001
SEI, mm^2*mm^{-2}	3.45[c]	3.70[b]	4.52[a]	0.04	<0.0001
Weight, g	0.28[b]	0.28[b]	0.32[a]	0.01	0.0067
Volume, cm^3	0.73[b]	0.79[a]	0.81[a]	0.02	0.0537
Density, $g*cm^{-3}$	0.38[ab]	0.35[b]	0.40[a]	0.01	0.0800
Kibble shrinkage, %					
Length	3.55	3.50	7.00	2.34	0.3125
Diameter	8.43[a]	6.58[b]	3.58[c]	1.26	0.0033
Kibble texture analysis					
Hardness, N	112.2[b]	146.0[a]	116.1[b]	8.09	0.0117
Compression energy, N*mm	6692[b]	10276[a]	3872[c]	493	0.0004

[abc] Means with unlike superscripts differ, $P < 0.05$.

[1] SEM: standard error of the mean.

Chapter 4 - The effects on nutrient utilization and stool quality of Beagle dogs fed diets with beet pulp, cellulose, and Miscanthus grass

Abstract

Dogs can benefit from dietary fibers. Traditionally, cellulose and beet pulp have been used by pet food companies as insoluble and soluble fiber sources. Miscanthus grass is a novel fiber ingredient made from Miscanthus giganteus, a C4 grass produced for its fiber content, but it has not been evaluated for dogs. The objectives of this study were to determine the effects of different fiber sources on nutrient utilization and stool consistency by dogs. Twelve Beagle dogs were fed three dietary treatments varying in their fiber sources (beet pulp - BP, cellulose - CE, Miscanthus grass - MG). Diets were fed for a 14-d period (9 d adaptation), fecal samples were collected (5 d total fecal collection) and scored. Nutrient digestibility was estimated using two different methods: total fecal collection (TFC) and chromic oxide (CRO). Dogs fed BP diet had softer stools than dogs fed CE and MG (3.15 vs. 3.68 and 3.64, respectively). Wet fecal output was higher for dogs fed CE compared to MG, with dogs fed BP having the lowest values (254.3 g vs. 241.6 g vs. 208.5 g, respectively). Both digestibility methods ranked dietary treatments similarly. Dogs fed CE and MG had lower dry matter (DM) digestibility than dogs fed BP ($P < 0.05$), dogs fed BP had lower crude protein (CP) digestibility compared to dogs fed MG and CE (81.4% vs. 85.5% and 85.8%, respectively). In conclusion, Miscanthus grass could be used as an alternative fiber source to cellulose.

Introduction

While pet owners may question the addition of fibers to a carnivore (dogs and cats) diet and make the choice to purchase diets dense in nutrients and energy, this may be detrimental to

their pets. This hypothesis is supported by the increasing numbers of overweight and obese animals in our homes. In 2007, 52% of dogs and 55% of cats were considered overweight or obese by their veterinarians compared to 56% and 60% in 2017 (APOP, 2018). Clearly the pet's energy expenditure is lower than their intake, and this excess energy can lead to fat deposition. Obesity is considered a disease by veterinarians which can negatively impact long-term animal health. For example, it can lead to joint, heart, metabolic, and endocrine issues, along with chronic inflammation (Kealy et al., 2002; German, 2006; Laflamme et al., 2006; German et al., 2009). Thus, weight control is a key factor to improve companion animal quality of life and overall longevity.

Pet food companies produce diets with reduced energy content that are intended for weight loss and management. They decrease calorie density by lowering fat and adding fibrous ingredients. Unlike starch in the diet, fiber is not digested by the animal's digestive enzymes; thus, it contributes little if any calories. Historically, cellulose has been the standard fiber source in low calorie diets (Burrows et al., 1982; de Godoy et al., 2013; Koppel et al., 2015). The ingredient "cellulose" is produced from trees in the process of making paper pulp. Several studies have shown that cellulose is poorly fermented (Sunvold et al., 1995ab), can decrease DM and OM digestibility in dogs (Muir et al., 1996), and increase fecal output (Wichert et al., 2002). Despite its benefits for caloric dilution, cellulose is expensive when compared to other fiber sources. Agricultural industries generate fibrous "wastes" as a result of producing human food ingredients. For example, these would include beet pulp, wheat bran, corn fibers, peanut hulls, rice bran, pea fiber, and others. Beet pulp is a prominent fiber used in pet foods. It is generated from the sugar beet industry after sugar is extracted for the sweeteners market. Beet pulp has been evaluated in dog foods and found to be moderately fermentable (Sunvold et al., 1995ab)

and resulted in better dry matter and organic matter digestibility compared to cellulose (Howard et al., 2000; Middelbos et al., 2007). However, CP and CFat digestibility declined slightly when beet pulp was added to the diet (Fahey et al., 1990ab; Muir et al., 1996; Sabchuk et al., 2017). However, fiber sources like beet pulp do not reduce the calorie content to the degree cellulose does. Since their soluble fiber content is much greater than cellulose, short chain fatty acids are produced through fermentation and used as energy by the animal (Hamer et al., 2008; Voet et al., 2016). These agricultural co-products have been successful in the pet food industry. The pet food market is in a constant search for new ingredients and discounts the use of byproducts. One potential fiber ingredient option is Miscanthus grass (*Miscanthus giganteus*) which is a C4 grass. Since the fiber of this grass is the intended product, it might be a well-accepted alternative to cellulose. Miscanthus grass has also been explored for application in cellulosic ethanol production (Adams et al., 2018), construction materials, paper-pulping, and as an absorbent (Visser and Pignatelli, 2001). However, to our knowledge, Miscanthus grass has never been evaluated for a pet food application. It was our hypothesis that Miscanthus grass could be an alternative to cellulose in dog foods. The objectives of this study were to determine the effects of different fiber sources on nutrient utilization and stool consistency by dogs.

Materials and Methods

Ingredients and Dietary Treatments

Dietary treatments were made from a similar base ration (90%) and one of three fiber sources at 10% inclusion (Table 4.1). Diets were formulated according to AAFCO (2015) nutrient profiles for adult dogs at maintenance and to be isonutritional with the exception of the fiber source contribution (Table 4.2). The base ration ingredients were sourced as a pre-blend from a commercial feed mill (Fairview Mills, Seneca, KS). The experimental fiber sources

included Miscanthus grass (Renew Biomass, Springfield, MO), cellulose and beet pulp (Fairview Mills, Seneca, KS).

To the pre-blend, fiber sources, chromium sesquioxide, and titanium dioxide were mixed in a paddle mixer (140 kg capacity) for 5 min. Dog foods were produced in a single screw extruder (model E525, Extru-Tech Inc., Seneca, KS). After extrusion, experimental diets were dried to less than 10% moisture in a convection oven. Then coated with chicken fat and flavor enhancer. Coated diets were stored in plastic bags in a temperature-controlled room (25ºC) for 7 d prior to the start of the feeding trial and nutrient analyses.

Feeding Trial and Sample Collection

The animal experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC protocol number 3645). Beagle dogs (12) were individually housed in metabolic cages (1.20 m x 1.83 m) in a room with controlled temperature (23ºC) and fresh water was available ad libitum throughout the duration of the study. Dogs were fed twice daily (8:00 and 16:30) and allowed 30 min to eat at each meal. Food allowance was controlled for the dogs to maintain body weight throughout the duration of the experiment.

Dogs were adapted to the test diet for 9 d and the following 5 d feces were collected. Dog body weight and body condition score (Laflamme, 1997) were recorded prior to, and at the end of each collection period. During the collection period, feces were collected twice daily, and defecation frequency was recorded. Feces were scored according to the following: 1 (liquid diarrhea) to 5 (hard pellets) with 3.5 considered ideal fecal score (Carciofi et al., 2008). After collection, fecal samples were frozen. A subsample of each experimental diet was collected weekly and then composited for further analyses.

Chemical Analysis

All sample analyses were performed in duplicates, with the exception of the TDF and insoluble fiber analyses, that were performed in triplicates. If the variation between the duplicates and among the triplicates was higher than 5%, the analysis was repeated. At the end of each collection period, fecal samples were thawed and placed in aluminum pans and dried to touch in a convection oven at 55ºC for 48 h. Food and fecal samples were ground (Retsch ZM200, Germany) to pass a 1-mm screen. Both food and fecal samples were analyzed for moisture (AOAC 930.15), crude protein (CP, AOAC 990.03), fat by acid hydrolysis (CFat, AOAC 954.02), ash (AOAC 942.05), gross energy by bomb calorimetry (bomb calorimeter model 1351, Parr Instrument Company, Moline, IL), and total dietary fiber (TDF, Prosky et al., 1985). In addition, the dog foods were analyzed for crude fiber (CFiber, AOAC 962.09). Fiber sources were analyzed for neutral detergent fiber (Van Soest and Wine, 1964), acid detergent fiber and acid detergent lignin (Van Soest, 1963), TDF, and insoluble and soluble fibers (Prosky et al., 1988).

To estimate digestibility, food and fecal samples were analyzed for chromium by atomic absorption as described by Williams et al. (1962) with adaptations. Briefly, 0.5 g of sample was ashed overnight at 600ºC. Cooled samples were digested on a hot plate with 3.0 ml of phosphoric acid manganese sulfate solutions and 4.0 ml of 4.5% potassium bromate solution. Once the sample was cooled to room temperature, 12.5 ml of 4,000 ppm calcium chloride solution was added, the sample was transferred to a sample cup and brought to 100 g with distilled water. After overnight rest, samples were analyzed for chromium at 357.9 nm by atomic absorption (Perkin Elmer 3110).

Digestibility Estimation

Nutrient digestibility was estimated using two different methods (total fecal collection – TFC, and chromium sesquioxide - CRO) using the equations bellow:

TFC= ((% ND*FI)-(%NF*FO))/ ((% ND*FI))

CRO= (1- (%CROD*%NF)/(%CROF*%ND)) *100

wherein: %ND is the percent nutrient in the diet, FI is the food intake in g, %NF is the nutrient in the feces, FO is the fecal output in g, %CROD is the percent chromium in the diet, and %CROF is the percent chromium in the feces.

Experimental Design and Statistical Analysis

This experiment was performed as a replicated 3x3 Latin Square design, wherein dog was the column factor, period the row factor, and diet was the treatment. Data was analyzed using statistical software via the general linear model procedure for mixed models (GLMMIX procedure in SAS; v. 9.4). The square, period and dog nested within square were considered as random factors. Fisher's least square means were considered different at alpha of 5% and trends were considered when the P-value ranged from 0.05 to 0.10. Additionally, the fecal score of each diet was tested against the ideal fecal score (3.5) using the TTEST procedure (SAS, v. 9.4). The mean fecal score was considered different than 3.5 when the P-value was smaller than 0.05.

Results and Discussion

Fiber-containing ingredients additions in dietary treatments were at 10% rather than an iso-TDF basis (Table 4.1). The thought behind the same inclusion was to have a high fiber content and a similar base line from the other ingredients of the diet. Thus, our theory was that the results reported would be due to the fiber source alone rather than differences from shifting the contribution from the other ingredients in the diet. Nutrient composition among diets was

similar (Table 4.2). As targeted in the production protocol the moisture for all diets was lower than the targeted 10%. Small variations among the dietary nutrient compositions were partially a result of the fiber sources. The CFiber and TDF content were lower for BP compared to MG. The CE diet had the highest CFiber and TDF contents. Crude fiber is measured by boiling the sample in a weak acid followed by boiling in a weak alkali (AOCS Ba 6a-05 method). Due to this sample digestion, most of the soluble fibers and a portion of the insoluble fibers are removed from the sample. Thus, the dietary fiber content of the sample is underestimated. While beet pulp has lower CFiber and TDF contents than Miscanthus grass and cellulose, the soluble fiber concentration is about three times higher for beet pulp compared to Miscanthus grass and 10 times higher compared to cellulose (Table 4.3). When considering the fiber profile of Miscanthus grass, clearly it is more similar to cellulose than beet pulp. This is a function of the raw materials and how Miscanthus grass is produced. Miscanthus grass is made from the dry canes of Miscanthus giganteus. The separation of the leaves from the canes is done during the winter, when the plant enters a dormant state, wherein nutrients from the leaves and canes are stored in the rhizomes and the dry leaves are dropped on the field. When the field dried canes are harvested, they are ground to produce a fibrous ingredient. Thus, there is an increase in the structural fiber content in the raw material, since the stems have higher concentration of cellulose than the leaves (Milic et al., 2011). This differs from cellulose which is chemically derived by the wood pulping process (Dhal, 1884). In this method wood chips are delignified and other insoluble and soluble fibers are solubilized and removed. Thus, the cellulose is concentrated and results in a higher insoluble fiber content when compared to Miscanthus grass (Table 4.3). Because Miscanthus grass is not processed through any purification steps, the lignin and soluble

fibers contents were higher than cellulose (Table 4.3). The fiber profile of both beet pulp and cellulose were similar to previous reports (Sunvold et al., 1995a, Jimenez-Moreno et al., 2013).

Food intake was similar among dietary treatments (average 236 g) and no refusals were observed throughout the duration of the feeding trial. Additionally, dog body weight and body condition score were maintained during the experimental procedure (average 10.56 kg and 5.21, respectively for body weight and body condition score; Table 4.4). Defecation frequency (average 2.96) was not affected by the type of fiber ingredient added to the diet. Fecal scores were similar for dogs fed MG and CE; however, dogs fed BP had softer stools. In addition to pairwise comparisons, each treatment mean was compared to the ideal fecal score (3.5) using a t-test. Dogs fed MG had a similar fecal score to the ideal. However, dogs fed BP had lower and CE had higher fecal scores than the targeted value ($P < 0.05$; Table 4.4). Wet fecal output was higher for dogs fed CE compared to dogs fed MG, with dogs fed BP having the least amount of feces. Fecal DM followed the same trend as wet fecal output (Table 4.4). As proposed previously, beet pulp is a moderately fermentable fiber source (Muir et al., 1996); therefore, a portion of the fiber is fermented and utilized by the microorganisms in the colon. The fermentation of the fiber produces short chain fatty acids (SCFA), and gasses (e.g. hydrogen, carbon dioxide, and hydrogen sulfide), which are either absorbed by the animal or expelled through flatulence (Yamka et al., 2006) and (or) in the breath (Felix et al., 2013). As a result, less organic material is excreted in the feces. Conversely, CE and MG diets had a higher concentration of insoluble fibers. This type of fiber is known to be non-fermentable (Sunvold et al., 1995ab). Thus, the more undigested and unfermented material is excreted by the dogs. Additionally, as fermentation takes place, complex molecular structures are being metabolized into smaller molecules by the colonic microbiome. With an increase in the fermentative end-

products there can also be an increase in water in the lumen. As undigested materials (e.g. fibers) are fermented to the other more soluble molecules (SCFA, lactic acid, CO_2, hydrogen gas, ammonia) an osmotic pull is created towards the lumen (Felix et al., 2013). Additionally, several of these substances are acids that at the luminal pH are ionized. Therefore, the luminal pH decreases over time due the transformation of soluble indigestible food components into SCFA, lactic acid and CO_2 (Biagi et al., 2010; Felix et al., 2013). As a response to the drop in pH, the colon may secrete more water (with bicarbonate) into the lumen to increase the pH and decrease any possible chemical irritation. Thus, an increase in fecal moisture is likely a reaction of the dog's colon to a combination of these two factors (osmotic pull and drop in pH). While this hypothesis still needs to be confirmed, an increase in fecal water and decrease in fecal pH due to microbial activity was reported by different researchers (Fahey et al., 1992; Guevara et al., 2008; Biagi et al., 2010; Felix et al., 2013; Panasevich et al., 2013; Silva et al., 2016). For example, Guevara et al. (2008) fed dogs diets containing beet pulp, and different types of corn fibers. These authors reported a decrease in fecal DM when beet pulp (moderately fermentable fiber) was added to the diet compared to the tested corn fibers. While the TDF content of these fiber sources were similar, the soluble fiber content of the corn fibers was much lower than the beet pulp (Guevara et al., 2008), thus supporting the hypothesis that the increase in soluble fibers in the colonic lumen may shift the water movement and decrease fecal dry matter. In addition to this shift in water movement in the colonic lumen, fiber sources have different water holding capacities, that could also contribute to decrease water absorption and fecal DM.

Nutrient and energy digestibility were estimated by two different methods: TFC and CRO (Table 4.5). In general, both digestibility methods separated the dietary treatments in a similar fashion; however, the standard error for CRO digestibility estimations was about twice as high as

the standard error for TFC. When considering the TFC method, dogs fed BP diet had higher DM and OM digestibilities compared to dogs fed MG, and dogs fed CE had the lowest DM and OM digestibilities (Table 4.5). Gross energy digestibility was higher for dogs fed BP than MG and CE (85.2% vs. 82.3% and 81.8%, respectively). These results could be explained by the higher content of fermentable fibers in BP compared to the other two test fibers. As these fibers are fermented in the colon, more energy is absorbed by the animal (SCFA, lactic acid) and converted into gasses (CO_2, H_2, methane, etc.); thus, less energy is eliminated as fecal material. The digestibilities of DM, OM, and GE are higher for fiber sources that have more fermentable fiber content. For example, Cutrignelli et al. (2009) used German Shepherd and Neapolitan Mastiff fecal inoculum and reported lower concentrations of acetate, propionate, and butyrate for pure cellulose compared to beet pulp. In that experiment, only 2.5% of organic matter disappeared for the pure cellulose, while for beet pulp it was 46.81%. Additionally, the maximum rate of fermentation of pure cellulose was about ten times lower compared to beet pulp. Similarly, in this experiment, both cellulose and Miscanthus grass had a higher concentration of insoluble (non-fermentable) fibers, thus dog fecal output was higher (Table 4.4) and DM, OM, and GE digestibilities were lower (Table 4.5). Conversely, CP and CFat digestibility were higher for dogs fed MG and CE than BP. Total dietary fiber digestibility was higher for dogs fed the BP diet compared to dogs fed the MG diet, and animals fed CE diet had the lowest TDF digestibility (63.0% vs. 46.1% vs. 37.5%, respectively; Table 4.5). The fermentation of the soluble fibers from the beet pulp may have two outcomes: increased fermentation end-products and microbial mass. Thus, as fermentation increases, more microorganisms are excreted by the animal and an underestimation of true digestibility is expected when fermentable fibers are present in the diet.

Similarly, Muir et al. (1996) reported a higher OM digestibility when dogs were fed BP diet compare to CE.

In addition to TFC, digestibility was estimated by CRO, which is a standard external marker for estimation of energy digestibility adopted by the American Association of Feed Controls Officials (2015). Dry matter, OM, and GE digestibility were higher for dogs fed BP compared to MG and CE (Table 4.5), whereas CP and CFat digestibilities were higher for dogs fed MG and CE compared to dogs fed BP. Dietary fiber digestibility was lowest for dogs fed CE diet and highest for dogs fed BP diet, with MG diet being intermediate (60.5% vs. 51.7% vs. 47.2%, respectively; Table 4.5). Silvio et al. (2000) fed dogs experimental diets varying in the proportion of insoluble and soluble fiber by changing the inclusions of cellulose and pectin and then measured digestibility at the ileum and total tract. They reported a decrease in fecal DM percentage as pectin content of the diet increased at the expense of cellulose, supporting the hypothesis that fermentation of soluble fibers could increase fecal water content. Yet, ileal DM digestibility was not affected by the insoluble to soluble fiber ratio of the diet. However, total tract DM digestibility increased as dietary cellulose was replaced by pectin. This is a good example that the fibers sources are responsible for changes in the DM, OM, GE, CP, CFat, and TDF total tract digestibility. Similar results were also reported by Cole et al. (1999) and Middelbos et al. (2007).

While the fiber composition of the diet has little impact on ileal digestibility, it can impact overall caloric content. Given that most of dogs in the U.S. are overweight (36.4%) and obese (19.6%; APOP, 2017), weight loss and management using insoluble non-fermentable fiber sources (e.g. Miscanthus grass and cellulose) could be an alternative to improve the health of those animals. Even though the fecal output may increase, the goal of decreasing the energy

intake would be met and there are no studies evaluating consumer's perception on fecal volume and (or) weight with the fiber content of the diet. Another benefit to the addition of dietary fibers to pet foods is to promote gut fill, improve satiety, and reduce begging and scavenging behaviors (Pappas et al., 1989). Fiber can also be used as a prebiotic. Pet foods targeting gut health gained popularity in recent years. In these diets soluble fermentable fibers are preferred (e.g. beet pulp). These fiber sources will serve as substrate for the microorganisms in the large intestine and stimulate fermentation. Butyrate, a fermentation end-product, is the preferred fuel source for the colonocytes (Bergman, 1990; Topping and Clifton, 2001), and has been considered a potential for prevention of colon cancer (Tungland and Meyer, 2002; Hamer et al., 2008). However, as detailed previously, with an increase in fermentation, luminal water content may increase, and feces could become softer. In this case, Miscanthus grass as an insoluble fiber source provided a good stool quality at a high level in the diet and reduced the energy intake by lowering energy digestibility, thus, it could be used as an alternative fiber source in dog foods.

Conclusion

Despite dogs being carnivores, they can benefit from fiber consumption for either weight management and (or) gut health. In general, dietary fiber decreases DM and OM total tract digestibility. The fiber composition of these three different fiber sources affected stool consistency and nutrient utilization differently. Dogs fed BP had softer stools and lower wet fecal weight and higher digestibility coefficients for DM, OM, GE and TDF, whereas dogs fed CE and MG had harder stools and higher CP and CFat digestibility. Despite the differences in ingredient composition between cellulose and Miscanthus grass, both fiber sources affected nutrient digestibility and stool quality in a similar fashion. Considering these results, Miscanthus grass could be an alternative fiber source to cellulose in dry extruded dog foods.

Author Contributions to the Chapter

RAD: experiment conduction, data and sample collection, sample analysis, statistical analysis, data interpretation, and manuscript preparation.

CGA: experiment design, data interpretation, and manuscript revision.

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Chapter 4 Tables

Table 4.1 Ingredient composition of experimental diets, as is basis.

Ingredient	Percentage
Fiber source	10.00
Chicken by-product meal, low-ash	29.96
Brewers rice	17.12
Corn	17.12
Wheat	14.55
Corn gluten meal	5.14
Titanium Dioxide	0.40
Chromium Sexquioxide	0.25
Potassium chloride	0.30
Salt	0.50
Choline chloride, 60%	0.23
Natural antioxidant	0.17
Vitamin premix [1]	0.15
Trace mineral premix [2]	0.10
Chicken fat [3]	3.00
Flavor enhancer [3]	1.00

[1] Vitamin E Supplement (79,887 IU*kg^{-1}), Niacin Supplement (64,736 mg*kg^{-1}), Calcium Pantothenate (12,186 mg*kg^{-1}), Vitamin A Supplement (17,162,998 IU*kg^{-1}), Thiamin Mononitrate (14,252 mg*kg^{-1}), Pyridoxine Hydrochloride (5,537 mg*kg^{-1}), Riboflavin Supplement (4,719 mg*kg^{-1}), Vitamin D3 Supplement (920,000 IU*kg^{-1}), Biotin (70 mg*kg^{-1}), Vitamin B12 Supplement (22 mg*kg^{-1}), Folic Acid (720 mg*kg^{-1}), as is basis.

[2] Zinc Sulfate (88,000 mg*kg^{-1}), Ferrous Sulfate (38,910 mg*kg^{-1}), Copper Sulfate (11,234 mg*kg^{-1}), Manganous Oxide (5,842 mg*kg^{-1}), Sodium Selenite (310 mg*kg^{-1}), Calcium Iodate (1,584 mg*kg^{-1}), as is basis.

[3] Added during the coating to the dried kibbles.

Table 4.2 Nutrient composition of experimental diets.

Composition	MG[1]	CE[1]	BP[1]
Dry matter	94.30	95.39	95.19
Crude protein*	31.02	29.09	29.89
Crude fat*	9.00	7.55	7.89
Ash*	6.10	5.99	6.84
Crude fiber*	6.01	8.24	3.74
Total dietary fiber*	19.97	20.47	17.59

[1] MG: Miscanthus grass diet, CE: cellulose diet, and BP: beet pulp diet.

* Dry matter basis

Table 4.3 Fiber fractions of dietary fiber source.

Composition*, %	Miscanthus grass	Cellulose	Beet pulp
Dry matter	95.00	95.30	92.53
Crude fiber	45.20	72.70	18.70
Acid detergent fiber	53.70	80.60	24.30
Neutral detergent fiber	73.80	88.40	31.60
Acid detergent lignin	13.00	0.70	5.90
Total dietary fiber	85.50	97.80	57.70
Insoluble fiber	78.60	95.30	33.30
Soluble fiber	6.90	2.50	24.40

*As is basis.

Table 4.4 Food intake (FI), defecation frequency (DF), fecal score (FS), wet fecal output (WFO), fecal dry matter, and t-test p-value from the comparison average fecal score = 3.5 of dogs fed diets with different fiber sources (N = 12).

Diet	MG[1]	CE[1]	BP[1]	SEM [2]	P-value
Body Weight, kg	10.60	10.56	10.53	0.41	0.4483
Body Condition Score	5.19	5.23	5.21	0.37	0.8858
Food Intake, g/d/dog	235.2	240.0	234.6	6.33	0.6529
Defecation Frequency, no/d/dog	2.98	3.03	2.88	0.16	0.6293
Fecal Score [3]	3.64[a]	3.68[a]	3.15[b]	0.06	<0.0001
Fecal Score = 3.5, P-value	0.4311	0.032	<0.0001		
Wet Fecal Output, g/d/dog	241.6[b]	254.3[a]	208.5[c]	6.44	<0.0001
Fecal Dry Matter, %	38.70[b]	40.94[a]	29.25[c]	0.52	<0.0001

[abc] Means with unlike superscripts differ (P < 0.05).

[1] Dietary treatments; MG: Miscanthus grass, BP: beet pulp, CE: cellulose

[2] SEM: Standard error of the mean

[3] Fecal score: 1 – liquid diarrhea, to 5 – dry hard pellets.

Table 4.5 Apparent total tract digestibility of dogs fed diets with varying fiber sources estimated by total fecal collection method (TFC) and Chromium sesquioxide (CRO).

Digestibility, %	MG[1]	BP[1]	CE[1]	SEM[2]	P-value
			TFC		
Dry Matter	78.2[b]	81.3[a]	77.2[c]	0.37	<0.0001
Organic Matter	82.1[b]	86.1[a]	80.8[c]	0.32	<0.0001
Gross Energy	82.3[b]	85.2[a]	81.8[b]	0.24	<0.0001
Crude Protein	87.9[a]	84.5[b]	87.6[a]	0.24	<0.0001
Crude Fat	90.7[a]	88.8[b]	90.9[a]	0.56	0.0099
Total Dietary Fiber	46.1[b]	63.0[a]	37.5[c]	0.72	<0.0001
			CRO		
Dry Matter	74.5[b]	78.6[a]	74.7[b]	0.75	0.0007
Organic Matter	78.6[b]	83.4[a]	78.1[b]	0.62	<0.0001
Gross Energy	78.8[b]	82.4[a]	79.2[b]	0.61	0.0006
Crude Protein	85.5[a]	81.4[b]	85.8[a]	0.52	<0.0001
Crude Fat	88.7[a]	86.6[b]	89.7[a]	0.72	0.0095
Total Dietary Fiber	51.7[b]	60.5[a]	47.2[c]	0.75	<0.0001

[abc] Means with unlike superscripts differ ($P < 0.05$).

[1] Dietary treatments; MG: Miscanthus grass, BP: beet pulp, CE: cellulose

[2] SEM: Standard error of the mean.

Chapter 5 - The effects of fiber source on extrusion processing and kibble macro and microstructure of dry cat foods

Abstract

Traditionally, cellulose and beet pulp have been used by pet food companies to increase dietary fiber content of cat foods. However, today's consumers are demanding alternative ingredient sources to by-products. Miscanthus grass may be an alternative; however, it has not been evaluated in pet foods. The objective of this study was to determine the effects of fiber sources on extrusion processing parameters and kibble macro and microstructure of cat foods. Cat foods containing 10% of Miscanthus grass (MG), cellulose (CE), or beet pulp (BP) were produced. Three batches of each cat food were separately mixed and extruded (model E535, Extru-Tech, Seneca, KS). During extrusion, feed rate, preconditioner water and steam, extruder screw speed, extruder water and steam addition, and knife speed were adjusted to achieve a wet bulk density of 330 $g*L^{-1}$. After extrusion, kibbles were dried in a convection oven (115.5ºC) until moisture was less than 10%. Then dried kibbles were coated with chicken fat and flavor enhancer. No effects due to fiber source were reported for extrusion parameters or kibble measurements ($P > 0.05$), with the exception of compression energy; wherein, kibbles produced with cellulose required more energy than those containing beet pulp (3917 N*mm vs. 3591 N*mm, respectively). In conclusion, process adjustments are minimal if Miscanthus grass is exchanged for cellulose or beet pulp in extruded cat foods.

Introduction

Obesity is an issue in the dog and cat population in the US. This disease is more common in cats than dogs, wherein 33.5% of cats are considered obese compared to 19.6% of dogs

(APOP, 2018). Historically, pet food companies have produced diets with lower caloric contents targeting this market. The dietary energy dilution, in most cases, has been accomplished by the reduction of fat and the addition of fiber. Since most fat is topically applied to extruded pet foods, this reduction is straightforward with no direct impact on the process. However, the addition of fiber can have deleterious effects on the quality of dry pet foods. This is because fibers are classified as disperse phase fillers (Guy, 1994) and are known to negatively affect expansion.

One of the most prominent fiber sources added to pet food for caloric restriction is cellulose from the paper pulping industry (Burrows et al., 1982; Koppel et al., 2015). However, this ingredient is costly compared to other dietary components. Beet pulp is another common fiber source studied in dog and cat foods for its effects on nutrient utilization (Fahey et al., 1990ab) and fermentation dynamics in vitro (Sunvold et al., 1995ab). Although nutritionally cellulose and beet pulp are fit for caloric restriction, consumer understanding of these ingredients does not align with their use by nutritionists and formulators. Thus, pet food manufacturers have been in search for alternative ingredients to differentiate their products and supply foods preferred by consumers. Miscanthus grass is a novel fiber source made from *Miscanthus giganteus*, a C4 grass. It is purposefully grown for its fiber content. Miscanthus grass has been previously tested by other industries (cellulosic ethanol; Adams et al., 2018, construction, paper pulping, and absorbent; Visser and Pignatelli, 2001). However, it has never been evaluated in a pet food matrix to the knowledge of the authors. Therefore, the hypothesis of this study was that Miscanthus grass would be a viable alternative fiber source to cellulose. The objective of this work was to determine the effects of Miscanthus grass, cellulose, and beet pulp on the extrusion processing parameters and kibble structure of cat foods.

Materials and Methods

Ingredient Sourcing, Diets Preparation, and Extrusion

Ingredients in the dry ration were pre-blended by a commercial feed mill (Fairview Mills, Seneca, KS), with the exception of the fiber sources, digestibility markers, chicken fat, and flavor enhancer. Cellulose and beet pulp were purchased from the same feed mill that provided the ration. Miscanthus grass was provided by Renew Biomass (Springfield, MO). Three individual batches of each cat food were blended to provide replication for each dietary treatment. Pre-blend, fiber source, chromic oxide, and titanium dioxide were mixed in a paddle mixer (140 kg capacity) for 5 min. Extrusion of the diets were performed on three separate days (one batch of each dietary treatment per day). The order that the diets were extruded was MG, CE, and BP on the first day; BP, CE, and MG on the second day; and BP, MG, and CE on the last extrusion day.

A single screw pilot scale extruder (model E525, Extru-Tech, Seneca, KS) was used for the production of the diets. The length:diameter ratio of the extruder was 13.1:1 with an internal barrel diameter of 133.35mm. Screw and barrel configuration were divided into seven sections (Figure 5.1). The feeding zone was the first section with a barrel without grooves and a screw with a forward single flight. For segments two through five, the screw was similar to the first section of the barrel; however, the barrel had a spiral groove to increase the retention time of the mixture in the extruder barrel. For the sixth section the barrel had a spiral groove and the screw was a 1.5 forward flight. For the last segment, a conical spiral groove barrel and conical double cut flight screw were used (Figure 5.1).

A wet bulk density of 330 $g*L^{-1}$ was targeted, and all extrusion parameters were adjusted to meet the desired wet bulk density. After steady state was achieved, feed rate, water and steam addition in the preconditioner, discharge temperature, extruder screw speed, water and steam

addition in the extruder barrel, die temperature, die pressure, knife speed, specific mechanical energy (SME, equation bellow), total mass flow, and wet bulk density were recorded every 20 min.

$$SME = \frac{\frac{\tau - \tau_0}{100} * \frac{N}{N_r} * P_r}{m}$$

wherein: SME is the specific mechanical energy in $kJ*kg^{-1}$, τ is the motor torque in N*m, τ_0 is the no load motor torque in N*m, N is the motor speed in rpm, N_r is the rated motor speed in rpm, P_r is the motor power in W, and m is the produced mass in kg. After extrusion, diets were dried in a convection oven at 115.5ºC until moisture was less than 10%. Chicken fat (4%) and flavor enhancer (1%) were applied as a coating after the diets were dried. After kibbles were coated, they were stored in paper bags with a plastic lining.

Kibble Macrostructure

In addition to the extrusion parameters, during each 20 min interval, 10 kibbles were collected after the extruder and were measured for their length and diameter (twice). Similarly, 10 kibbles out of the dryer were measured for length and diameter (twice) and weighed for calculation of piece volume, density, and sectional expansion ratio index (SEI) as follows:

$$V = \frac{\pi * h * D_k^2}{4}$$

$$d = \frac{m_k}{V}$$

$$SEI = \frac{D_k^2}{D_d^2}$$

wherein: V is the volume in mL, h is the kibble length in mm, D_k is the average of the two measurements of the kibble diameter in mm, d is the kibble density in $g*L^{-1}$, m_k is the kibble mass in g, SEI is the sectional expansion ratio index, and D_d is the die hole diameter in mm.

Texture Analysis

Texture analysis was performed using a texture analyzer (model TA-XT2, Texture Technology Corp., Scarsdake, NJ) equipped with a 50 kg load cell. A cylindrical probe (25 mm diameter) was used to compress 30 kibbles from each collection point for each batch (total 90 kibbles per day per diet). Kibbles from each time point and collection point were conditioned in a convection oven at 45ºC for 48h and then moved into a desiccator for 24h at room temperature to allow for moisture to be equilibrated among treatments. The pre-test speed was 2mm*s^{-1}, test speed was 1mm*s^{-1}, and a post-test speed was 10mm*s^{-1} (adapted from Dogan and Kokini, 2007). Strain level was set at 90%. Kibble hardness (N) was considered to be the peak force of the first major kibble breakage, the energy to compress (N*mm) the kibbles to 90% was the computed area under the curve for each compressed kibble. Negative values were rounded to zero for the calculation of energy to compress. The average values of 90 kibbles for hardness and compression energy were used as an experimental unit for statistical analysis. This high number of kibbles was used to account for the variable kibble size.

Chemical and Physical Analysis

Each experimental fiber source was analyzed for its geometric mean diameter (DGW ± S_{gw}; ASABE, 2008; method S319.4) in duplicates, calculated as follows:

$$DGW = 10^{\frac{\sum(W_i * 0.5 * \log(d_{i-1} * d_i))}{\sum W_i}}$$

$$Sgw = 0.5 * DGW * (10^{S_{log}} - \frac{1}{10^{S_{log}}})$$

wherein: W_i is the sample weight, d_i is the nominal sieve aperture size of the i^{th} sieve, d_{i-1} is the nominal sieve aperture size of the $i\text{-}1^{th}$ sieve, S_{gw} is the standard deviation, and S_{log} is the geometric standard deviation of log-normal distribution by mass in base-ten logarithm calculated as follows:

$$S_{log} = \left(\frac{\sum(W_i * ((0.5 * \log(d_{i-1} * d_i)) - \log(DGW))^2}{\sum W_i}\right)^{0.5}$$

The bulk density (g*mL^{-1}) of the fiber sources was measured in triplicate. Briefly, a 100 mL graduated cylinder was filled until the 100 mL mark, then, it was gently shaken to remove any bridges inside the cylinder and refilled until 100 mL if necessary. Next the cylinder with the fiber was weighed (Sudha et al., 2007). Bulk density was calculated as follows:

$$Bulk\ Density = \frac{W}{100}$$

wherein: W is the weight of the fiber in the cylinder. The average of the three measurements for each fiber source are reported (Table 3).

In addition to the physical characterization of the fiber sources, fiber sources were analyzed for their fiber contents by reference methods (Van Soest, 1963; Van Soest and Wine, 1964; Prosky et al., 1985; Prosky et al., 1988; AOCS Ba 6a-05; Table 3). Experimental diets were analyzed for their nutritional composition and moisture according to standard methods (moisture AOAC 930.15; crude protein AOAC 990.03; fat by acid hydrolysis AOAC 954.02; ash AOAC 942.05; total dietary fiber; and crude fiber AOCS Ba 6a-05) and gross energy content by bomb calorimetry (model 1351, Parr Instrument Company, Moline, IL). Samples were analyzed in duplicates, with the exception of the total dietary fiber analysis that was performed in triplicates. Sample analysis was repeated if the variation between the duplicates was higher than 5%.

Experimental Design and Statistical Analysis

A complete block design was used as the experimental design. Day was considered the blocking factor and batch was considered as the experimental unit. The general linear model from the mixed procedure was used to analyze the data (GLIMMIX procedure, SAS, v. 9.4, SAS Institute, Inc., Cary, NC), and the blocking factor was considered a random factor. Least square

means were considered different at alpha of 5%. Additionally, a t-test was used to evaluate if each treatment average wet bulk density against the targeted wet bulk density ($330g*L^{-1}$). Treatments were considered different from target if P was smaller than alpha ($\alpha < 0.05$), and trends were considered when P varied from 0.05 to 0.10.

Results and Discussion

Nutrient composition of dietary treatments was comparable to the predicted composition (Table 5.2). The fiber composition of cellulose, beet pulp, and Miscanthus grass used in this study were reported on table 3.3. The crude fiber composition tracked in a similar fashion to these experimental ingredients; whereas, the crude fiber content of BP was the lowest among all foods (2.95%). Beet pulp is known to contain moderate amounts of soluble fibers (Fahey et al., 1990a). These soluble fibers, and some insoluble fibers, are solubilized during the analysis for crude fiber resulting in an underestimation of the dietary fiber content, and this likely occurred in this case. As expected from previous analyses Miscanthus grass diet had an intermediate crude fiber concentration (5.56%) compared to BP and CE, whereas CE had the highest crude fiber content of all treatments (8.90%). This result is likely due to the high recovery rates of cellulose by the crude fiber analysis. Although crude fiber is a required item on the pet food labels according to the American Association of Feed Control Officials (AAFCO, 2015), this method underestimates the nutritionally relevant fiber content of the food. Thus, it would be more representative to characterize the fiber by methods that fully describe the fiber composition (e.g. total dietary fiber) for a given ingredient or pet food. Total dietary fiber of all diets was higher than the crude fiber content (Table 5.2). This method of analysis is based on enzymatic digestion of carbohydrates and proteins. Then, the soluble fibers are precipitated by ethanol and recovered with the insoluble fibers during a filtration step. The fiber concentration is then calculated after

corrections for the protein and ash of the residue retained on the filter. This method can be considered more accurate than crude fiber, because it would account for the fibers that are solubilized in both acid and base. In addition to more accurate fiber values, this characterization of the food allows for a better understanding of the dietary benefits for the nourishment of the animal. For example, a diet rich in insoluble fibers could aid in weight and hairball management (Fahey et al., 1990; Davenport et al., 2008; Loureiro et al., 2017), while a diet with a higher concentration of soluble fibers could benefit gut health (Sunvold et al., 1995b).

As previously highlighted, cellulose and beet pulp are two fiber standards used by the pet food industry. Cellulose is a source of insoluble fiber, while beet pulp has a considerable amount of soluble fibers (Table 5.3). Miscanthus grass, despite the high content of insoluble fibers, has a higher amount of soluble fibers than cellulose. This difference in these ingredients is mainly due to their manufacturing process. Cellulose is derived from the paper and pulp industry, after having been processed through several purification steps (Dahl, 1884). Essentially, wood chips are digested and cellulose from the tree cell walls is separated from the other cellular components and recovered in further steps. Conversely, Miscanthus grass is deliberately produced for its fiber content. This grass, which can grow in excess of 3 m tall, dries in the field during the winter, and the leaves drop onto the field. The dried canes are then harvested and ground to customer specifications. Since the raw materials for the production of this ingredient do not pass through any purification step (like cellulose) the concentration of soluble fibers and lignin remain and are therefore higher than cellulose (Table 5.3). Beet pulp is the residue from the removal of sugar from beets for the sweetener market. However, the soluble fiber content in Miscanthus grass is about a quarter to a third of that of beet pulp.

During the production of these cat foods, a targeted wet bulk density of 330 $g*L^{-1}$ was chosen based on our previous experiences. As reported in Table 5.4, the fiber source did not have any effect on the parameters evaluated during extrusion ($P > 0.05$). When comparing the wet bulk density of each individual treatment, only the BP diet tended ($P = 0.0833$) to be less dense than the targeted value (314 $g*L^{-1}$ vs. 330 $g*L^{-1}$). Conversely, the wet bulk density of the MG and CE diets were on target (Table 5.4). Despite the BP diet having numerically greater bulk density than the other two diets, the standard deviation of the replicates was lower when compared to the deviation for the MG and CE diets (8.54 $g*L^{-1}$ vs. 15.28 $g*L^{-1}$ and 22.12 $g*L^{-1}$, respectively); thus, the higher variation observed in the MG and CE cat foods could partially account for these results. Since there is very limited literature regarding the extrusion of pet foods (especially cat foods), reports from human foods (expanded extruded snacks and breakfast cereals) may provide context. Although the process to make these foods is similar, caution must be taken when comparing the results. First, it is important to note that the complexity of a pet food formula is much greater than a breakfast cereal or snack. The higher protein, fat, ash, and fiber content of pet foods have an impact on the process, especially when considering their combined effects. Several authors have evaluated the impact of each one of these components on extrusion processing for different human foods (Alam et al., 2014; Wang et al., 2017). In this particular study, the fiber component of the product will be the focus of the discussion since all treatments had the same ingredient composition and inclusion with the exception of the fiber source.

The tendency toward a less dense product could also be related to the kibble structure. As reported by other studies the addition of fiber to extruded starch matrices generally results in decreased expansion (Wang et al., 2017). However, this decrease is dependent on the fiber type,

inclusion level, and particle size. Soluble fibers, for example, become part of the starch matrix as they solubilize during extrusion. However, they do not form as strong a structure as starches, resulting in a decrease in hardness and energy to deform. For example, the addition of pectin to extruded corn starch decreased hardness compared to a control without pectin (Yanniotis et al., 2007). Conversely, insoluble fibers do not mix with the melt. Thus, weak spots in the matrix are created and premature cell rupture occurs. As a result, from this premature bursting, the kibbles become less expanded, harder, and more energy is needed for compression during analysis (Alam et al., 2014; Kallu et al., 2017). However, Wang et al. (2017) evaluated different inclusions and particle sizes of cellulose into expanded corn starch and concluded that at lower levels (5%), finely ground cellulose (<125 μm) was able to improve expansion by providing more nucleation points for the formation of air cells. With a change in expansion, other measurements of the kibbles can also be affected. For example, radial expansion decreases as insoluble fiber is added to the diet. This may be a result of insoluble fiber particles aligning with the flow of extrudate passing through the diet openings. This alignment promotes some strengthening of the cell walls to force a longitudinal expansion rather than radial (Brennan et al., 2008). Additionally, as kibbles expand less, they have a smaller volume and may become denser. In this study, none of the kibble measurements were affected by dietary fiber source ($P > 0.05$), with the exception of energy to deform. In that case, kibbles from CE diet needed more energy than kibbles from BP diet to be compressed (6917 N*mm vs. 3591 N*mm, respectively). The similar results in most of the evaluated parameters was likely not just a response of the fiber effects, but rather a combination of factors. Fibers are known to impact starchy extruded products; however, in this case it is important to highlight that the starch in the formula was diluted by more than just the fiber sources. About 57% of these formulas were composed of

ingredients that have no structure forming properties, that act as lubricants and (or) dispersed phase fillers (fiber, chicken byproduct meal, corn gluten meal, fats, minerals, vitamins, antioxidants). Therefore, when considering the formula as a whole, the dilution of starch and the high inclusion of fiber may explain why these different fiber sources produced a similar product with respect to kibble length and diameter, SEI, weight, volume, density, and hardness, and could have been a shortcoming of this experiment. The only parameter affected was compression energy, in which the CE kibbles required more energy to compress than the BP kibbles. In this case, the insoluble fiber content of cellulose may have further increased cell wall thickness, which, in turn, increased the energy required to compress the kibble. As reported by Yanniotis et al. (2007), as dietary insoluble fiber increased, expansion decreased, and hardness increased. As a result, an increase in compression energy is expected because the cell wall thickness increases due to premature cell rupture. As the cell does not fully expand due to the weak spots caused by the fiber particles, the walls become thicker. Thus, more energy is necessary to break the walls and compress the kibble during the analysis.

In summary, with respect to the fiber composition, Miscanthus grass has a high insoluble and low soluble fiber content; therefore, it is more similar to cellulose than beet pulp. Regardless of fiber ingredient composition, no effects due to fiber source were reported on extrusion processing of cat foods and kibble measurements, with the exception of the compression energy, wherein CE kibbles needed about twice the energy of the BP kibbles to be deformed. These differences in compression energy may be related to the high insoluble fiber content of cellulose, that caused premature burst of cell, thickened the cell walls, thus requiring more energy for compression.

Conclusion

In conclusion, the higher content of ingredients that do not contribute to structure formation may have limited the effects of the tested fiber sources and may be partially responsible for these results. Despite the lack of differences between treatments, Miscanthus grass has a fiber composition similar to that of cellulose: high insoluble fiber, fine particle size, and low density. Therefore, it could be used as an alternative fiber source to cellulose in cat foods without a need to changes in processing conditions to produce a similar product.

Author Contributions to the Chapter

RAD: experiment conduction, data and sample collection, sample analysis, statistical analysis, data interpretation, and manuscript preparation.

CGA: experiment design, data interpretation, and manuscript revision.

HD: texture analysis.

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Chapter 5 Figures

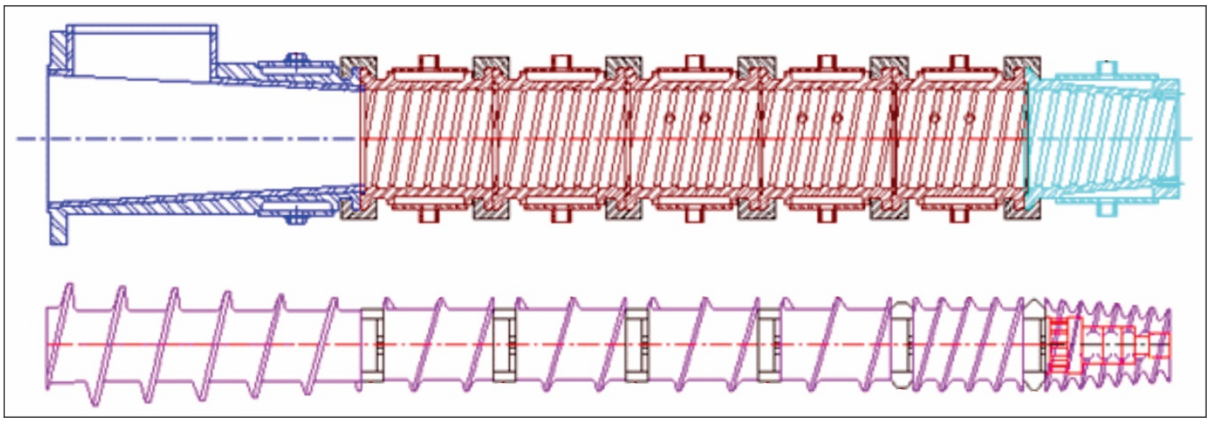

Figure 5.1 Screw and barrel profile of E525 extruder. Inlet on the left side, outlet on the right.

Chapter 5 Tables

Table 5.1 Ingredient composition of experimental diets, which were mixed and ground to pass a US number 16 sieve prior to extrusion.

Ingredient	Percentage
Fiber source	10.00
Ration	84.34
Chicken byproduct meal low ash	35.22
Brewers rice	14.07
Corn	14.07
Wheat	14.07
Corn gluten meal (75%CP)	5.00
Salt	0.40
Potassium Chloride	0.26
Choline Chloride (60%)	0.20
Vitamin Premix [1]	0.20
Calcium Carbonate	0.20
Trace Mineral Premix [2]	0.20
Fish Oil	0.10
Taurine	0.10
Natural AOX	0.10
Titanium oxide	0.40
Chromium sesquioxide	0.25
Chicken fat*	4.01
Flavor enhancer*	1.00

*Added after the diets were dried to less than 10% moisture

[1] Vitamin E Supplement (79,887 IU*kg^{-1}), Niacin Supplement (64,736 mg*kg^{-1}), Calcium Pantothenate (12,186 mg*kg^{-1}), Vitamin A Supplement (17,162,998 IU*kg^{-1}), Thiamin Mononitrate (14,252 mg*kg^{-1}), Pyridoxine Hydrochloride (5,537 mg*kg^{-1}), Riboflavin Supplement (4,719 mg*kg^{-1}), Vitamin D3 Supplement (920,000 IU*kg^{-1}), Biotin (70 mg*kg^{-1}), Vitamin B12 Supplement (22 mg*kg^{-1}), Folic Acid (720 mg*kg^{-1}), as is basis.

[2] Zinc Sulfate (88,000 mg*kg^{-1}), Ferrous Sulfate (38,910 mg*kg^{-1}), Copper Sulfate (11,234 mg*kg^{-1}), Manganous Oxide (5,842 mg*kg^{-1}), Sodium Selenite (310 mg*kg^{-1}), Calcium Iodate (1,584 mg*kg^{-1}), as is basis.

* Added during the coating to the dried kibbles.

Table 5.2 Nutrient composition and gross energy content of dietary treatments (dry matter basis) produced to evaluate processing parameters during extrusion and after drying.

Composition	MG[1]	CE[1]	BP[1]
Dry matter	94.53	94.48	94.60
Crude protein*	35.40	34.20	33.80
Crude fat*	11.40	12.00	11.60
Ash*	7.16	7.01	7.00
Crude fiber*	5.56	8.90	2.95
Total dietary fiber	13.76	14.48	10.88
Gross energy, kcal*kg^{-1}	4839	4823	4839

*Dry matter basis.
[1] MG: Miscanthus grass, CE: cellulose, BP: beet pulp.

Table 5.3 Test fiber source composition and physical characteristics.

Composition	MG[1]	CE[1]	BP[1]
Dry matter, %	95.00	95.30	92.53
Crude fiber, %	47.58	76.29	20.21
Acid detergent fiber, %	56.53	84.58	26.26
Neutral detergent fiber, %	77.68	92.76	34.15
Acid detergent lignin, %	13.68	0.73	6.38
Total dietary fiber, %	90.00	102.62	62.36
Insoluble fiber, %	82.74	100.00	35.99
Soluble fiber, %	7.26	2.62	26.37
Bulk Density, $g*mL^{-1}$	0.31	0.19	0.73
DGW ± S_{gw}[2], µm	103.46 ± 76.39	77.33 ± 44.47	193.78 ± 194.83

[1] MG: Miscanthus grass cat food, CE: cellulose cat food, BP: beet pulp cat food.
[2] DGW = Geometric Mean Diameter, S_{gw} = Standard Deviation; ASABE, 2008.

Table 5.4 Processing conditions of cat foods with different fiber sources.

Parameter	MG[1]	CE[1]	BP[1]	SEM[2]	P-value
Feed rate, kg*h^{-1}	239	250	239	6.56	0.4444
Preconditioner					
Water, kg*h^{-1}	41.3	37.6	38.0	2.04	0.4495
Steam, kg*h^{-1}	29.4	29.6	27.7	1.42	0.3443
Temperature, ºC	67.7	67.0	65.4	3.00	0.5050
Extruder					
Screw speed, rpm	369	336	350	18.14	0.4053
Water, kg*h^{-1}	0.00	0.00	0.00		
Steam, kg*h^{-1}	17.3	22.5	16.6	5.66	0.4465
Die					
Temperature, ºC	145.1	144.6	145.3	1.62	0.9516
Pressure, psi	350	350	350		
Knife speed, rpm	1650	1694	1827	226	0.6462
Other					
Specific Mechanical Energy, J*kg^{-1}	141.3	143.2	152.0	6.54	0.3636
Total Mass Flow, kg*h^{-1}	289	298	286	7.83	0.4009
Diet Wet Bulk Density, g*L^{-1}	306	313	314	9.40	0.7230
Bulk density = 330 g*L^{-1}, P-value	0.1994	0.1970	0.0833		

[1] MG: Miscanthus grass, CE: cellulose, BP: beet pulp.
[2] SEM: standard error of the mean.

Table 5.5 Kibble characteristics out of the extruder and drier, shrinkage, and macrostructure of dry dog foods with varying fiber sources.

Kibble parameter	MG[1]	CE[1]	BP[1]	SEM[2]	P-value
	Out of the extruder				
Length, mm	7.57	8.04	6.20	0.49	0.1074
Diameter, mm	7.87	8.32	7.67	0.40	0.4116
	Out of the drier				
Length, mm	6.77	7.49	5.73	0.51	0.1616
Diameter, mm	6.75	7.10	7.12	0.43	0.7331
SEI, mm^2*mm^{-2}	3.58	3.92	3.95	0.45	0.7500
Weight, g	0.126	0.118	0.131	0.0067	0.4579
Volume, cm^3	0.254	0.297	0.235	0.047	0.6162
Density, $g*cm^{-3}$	0.593	0.400	0.597	0.109	0.3709
	Kibble texture analysis				
Hardness, N	6.18	5.87	7.55	0.55	0.1380
Compression energy, N*mm	5145[ab]	6917[a]	3591[b]	693.78	0.0287

[ab] Means with unlike superscripts differ, $P < 0.05$.
[1] MG: Miscanthus grass, CE: cellulose, BP: beet pulp.
[2] SEM: standard error of the mean.

Chapter 6 - The effects on nutrient utilization, stool quality, and hairball management of cats fed diets varying in fiber sources

Abstract

To meet the specific needs of animals, pet food companies have produced diets such as weight management and hairball control diets. These conditions are particularly common in cats. Different fiber sources have been utilized in such diets to aid in the management of these animal issues. However, pet food companies seek alternative ingredients to differentiate their products from competitors. Miscanthus grass could be an alternative fiber source for pet food companies since it is novel. The objectives of this work were to determine the effects of Miscanthus grass as a fiber source on nutrient utilization, stool quality, and hairball management in cats. Dry extruded cat foods were produced for two feeding trials. Cats were fed experimental diets for a 9-d adaptation period followed by a 5-d total fecal collection period. In general, the cats fed the beet pulp diet (BPD) had higher dry matter, organic matter, gross energy, and total dietary fiber digestibility than cats fed Miscanthus grass (MGD) or cellulose (CED) diets ($P < 0.05$). However, crude protein digestibility was lower for cats fed BPD (82.1 vs. 84.7 and 85.1%, respectively for BPD, MGD, and CED). Fecal scores were lower for cats fed BPD (2.84) compared to MGD (3.32) and CED (3.21; $P < 0.05$). No effects due to fiber were reported on the fecal hairball variables, with the exception of less total hair weight and hair masses per gram of dry feces for cats fed Miscanthus grass (MGH) compared to control diet (COH; $P < 0.05$). In conclusion, Miscanthus grass could be used as an alternative ingredient to cellulose in cat diets.

Introduction

Obesity amongst the pet population is a growing issue. This issue is more prominent in cats than dogs with 33.5% of the US cat population obese versus 19.6% of the US dog population (APOP, 2018). To address this issue, pet food companies have produced diets with reduced caloric content. This reduction is often achieved by a higher inclusion of fiber. Traditionally, cellulose from the paper and pulp industry, and beet pulp from the sugar industry are used as standard fiber sources. The ingredient "cellulose" is a purified material, made after the digestion of wood chips and purification of the plant (tree) cell wall which is bleached to produce a white powder (Dahl, 1884). Beet pulp is the leftover fibrous material from the extraction of sugar from beets. Despite the nutritional benefits of both fiber sources (Fahey et al., 1990ab; Sunvold et al., 1995ab), they are not well accepted by pet food companies. Due to these aversions to by-product ingredients, many pet food companies are searching for novel ingredients to use as an alternative to these traditional ingredients.

In addition to the higher percentage of obese cats when compared to dogs, cats are also known to suffer from hairball regurgitation. While some people may think this is a normal condition, it can create other complications, such as hiatal hernias (Owen et al., 2005), intestinal blockages (Barrs et al., 1999), or fresh blood in the feces and colonic mucosa inflammation (Cannon, 2013). Some authors have tested different fiber sources, either included in the diet or supplemented in the meal, to aid the passage of hairballs from the stomach to the intestine. The methodologies, fiber sources, and results have varied greatly. Work reported by Lewis and Heaton (1999) showed that supplementation of plastic flakes could increase passage rate in human subjects; perhaps in a similar manner the addition of an insoluble fiber to foods might also provide the stimulus necessary to move hairballs through to the intestine.

Miscanthus grass is a fibrous ingredient produced from the dried canes of *Miscanthus giganteus*. Different than cellulose and beet pulp, the fibrous portion of *M. giganteus* is the main product of this crop; thus, a fibrous ingredient produced from this grass would not be perceived as a by-product by consumers and might provide an alternative to traditional fiber sources. Other uses for Miscanthus have been explored such as cellulosic ethanol production (Adams et al., 2018), construction materials, paper-pulping, and as an absorbent (Visser and Pignatelli, 2001). However, this ingredient has not previously been tested in cat foods. Therefore, the objective of this study was to determine the effects of Miscanthus grass in cat foods on nutrient digestibility and stool quality, and effects on hairball management when compared to cellulose and beet pulp containing diets.

Materials and Methods

Ingredients and Dietary Treatments

For the digestibility experiment diets containing three different fiber sources [cellulose (Fairview Mills, Seneca, KS), beet pulp (Fairview Mills, Seneca, KS), Miscanthus grass (Renew Biomass, Springfield, MO)] were formulated to be isonutritional and contain equal amounts of the fiber sources (Table 6.1).Titanium dioxide and chromium sesquioxide were added to serve as external fecal markers in order to measure fecal output and to calculate estimated nutrient utilization. Three batches of each diet were mixed separately in a paddle mixer (140 kg capacity). For the production of the diets, a single screw extruder (model E525, Extru-Tech, Seneca, KS) was used. Extrusion took place over three different days; wherein, one batch of each experimental diet was processed in each production day. After extrusion, kibbles were dried in a convection oven at 115.5ºC until the moisture was less than 10%. Fiber sources were analyzed for their fiber content by standard methods (moisture, AOAC 930.15; crude fiber, AOAC

962.09; acid detergent fiber and acid detergent lignin, van Soest, 1963; neutral detergent fiber, van Soest and Wine, 1964; total dietary fiber, Prosky et al., 1985; insoluble and soluble fiber, Prosky et al., 1988). Physical characterization of fiber sources was evaluated using two different methods: bulk density (Sudha et al., 2007) and particle size (ASABE, 2008; method S319.4).

For the hairball experiment two dietary treatments were produced: control (COH) and Miscanthus grass (MGH). The inclusion level of Miscanthus grass was 10% and it was replaced by rice flour in the control diet at the same concentration (Table 6.2). Extrusion and drying conditions were similar to the diets for the digestibility study. Flavor enhancer was added at 1.5% to ensure better palatability and avoid food refusals.

Digestibility Study

The experimental procedure for the digestibility trial was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC protocol number 3669). For this study, 12 American shorthair cats were used (8 males and 4 females, average weight 4.59 kg). Cats were evaluated for weight and body condition score (Laflamme, 1997) at the beginning and end of each collection period. Food allowance was controlled for the cats maintain body weight throughout the duration of the experiment. Each collection period was composed of 9 d of adaptation, wherein the cats were group housed but fed individually. During the adaptation phase, cats were fed at 8:00 and 16:30 with access to food for 1 h. During the adaptation periods some of the cats refused to eat the experimental diets. In those cases, an additional 0.5% of flavor enhancer was added topically to the food and was sufficient to stimulate food consumption. After the 9 d of adaptation, the cats were housed individually in stainless steel cages for 5 d of total fecal collection. During the collection phase, food was provided at 8:00 and 16:30 with 1 h access to food and feces were collected daily before and after the feeding. Orts were collected

and weighed to compute food intake. After collected, fecal samples were scored on a 1-5 scale in 0.5 increments (1- liquid diarrhea to 5 – dry hard pellets; Carciofi et al., 2008) and stored in a plastic bag in a freezer until further processed.

At the end of each collection period, wet fecal samples were weighed and placed in a aluminum pan to thaw. Once thawed, samples were placed in a convection oven at 55ºC until dry to touch, and sample weight was recorded. Next, the samples were ground to pass a 1-mm screen using a lab scale grinder (Retsch ZM200, Germany). Nutrient utilization was estimated by total fecal collection (TFC) and titanium dioxide (TIO) using the following equations:

TFC= ((%ND*FI)-(%NF*FO))/((%ND*FI))

TIO=(1 - (%TIOD*%NF)/(%TIOF*%ND))*100

wherein: %ND is the percent nutrient in the diet, FI is the food intake in g, %NF is the percent nutrient in the feces, FO is the fecal output in g, %TIOD is the percent titanium in the diet, and %TIOF is the percent titanium in the feces. For the digestibility estimation, fecal and food samples were analyzed for moisture (AOAC 930.15), ash (AOAC 942.05), gross energy (bomb calorimetry, model 1351, Parr Instrument Company, Moline, IL), crude protein (AOAC 990.03), crude fat by acid hydrolysis (AOAC 954.02), crude fiber (diets only; AOAC 962.09), and total dietary fiber (Prosky et al., 1985). Samples were analyzed in duplicates, with the exception of TDF; whereas, triplicates were analyzed. If the variation between duplicates was higher than 5%, the sample analysis was repeated.

For the estimation of nutrient utilization by titanium dioxide (Leone, 1973 with adaptations) 0.3 g of fecal sample or 0.6 g of food sample were ashed overnight in muffle furnace at 450ºC and allowed to cool to room temperature. Next, 1.0 g of sodium sulfate and 5 mL of sulfuric acid were added to the sample. The samples were digested at 280ºC for 25 min.

After being cooled to room temperature, the samples were transferred to 50 mL centrifuge tubes and brought to 50 g with distilled water. The tubes were centrifuged at 1,000 x G for 10 min and allowed to rest for 24 h. Next, 0.25 mL of sample was pipetted into a 96-well plate (in duplicate) for each sample. Then 30 µL of 30% hydrogen peroxide was added to each well, and the plate was allowed to rest on the counter for 15 min. Finally, absorbance was measured at 410 nm in a plate reader (Gen5TM, Biotek® Instruments, Inc.Winooski, VT).

Hairball Trial, Sample Collection, and Sample Processing

The experimental procedure for the hairball trial was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC protocol number 3785). Twelve American shorthair cats (8 males and 4 females) were fed experimental diets for 16 d adaptation. During this period the cats were group housed, but individually fed in metabolic cages at 8:00 and 16:30, with food available for 1 h. The following 5 d the animals were individually housed in stainless steel cages, fed, and feces were collected. Fecal samples were stored in plastic bags and frozen until processed. During this evaluation one of the cats refused to eat even with the addition of additional flavor enhancer and was removed from the trial.

The cats were brushed with 100 strokes of a brush designed for short hair cats (Furminator®, Blacksburg, VA) before the start of the experiment and at the end of each experimental period. Before sample processing feces were weighed. A sub-sample of feces was collected and analyzed for moisture (AOAC 930.15). The remainder of feces were placed into an aluminum pan and soaked overnight in water to soften the material and facilitate the separation of the hair mass without disrupting the entangled hair clumps. Next, the water was drained by pouring the sample and water into a wire mesh strainer. The remaining fecal material was removed with water using a squirt bottle. After the hair clumps were separated from the feces,

they were measured for length and diameter using a digital caliper. Once measured, each mass was placed in a pre-weighed aluminum pan for determination of dry hair weight by oven drying at 105ºC overnight. The remaining hair in the strainer was placed in an aluminum pan, dried, and weighed as previously described for the hair clumps. This weight was considered the amount of hair that was mixed with the feces rather than in a hair clump. Fecal hair clumps were classified according to their size as follows: extra small (<10.0 mm x <5.0 mm; length x diameter), small (10.0 - 20.0 mm x 3.5 - 6.5 mm), medium (20.0 - 30.0 mm x 4.0 - 7.0 mm), large (30.0 - 40.0 mm x 4.5 - 8.5 mm), and extra-large (>40.0 mm x >5.0 mm). Just one hairball was regurgitated by one cat, thus, no statistical analysis was performed for hairball incidents; this cat was on the control diet. In addition to the hair clumps characterization, diets were analyzed for total dietary fiber (Prosky et al., 1985) and insoluble fiber (Prosky et al., 1988). Soluble fiber content was estimated by subtracting the insoluble fiber content from the total dietary fiber (Fahey et al. 2019).

Experimental Design and Statistical Analysis

The digestibility experiment was performed as a replicated 3x3 Latin Square design, wherein the row factor was the dietary treatments and the column factor was the cats. Data was analyzed using statistical software via the general linear model procedure for mixed models (GLMMIX procedure in SAS; v. 9.4). The square, period, and cat nested within square were considered as random factors. In addition, a t-test (TTEST procedure, SAS, v. 9.4) was used to test if the treatment means for fecal scores were similar to the ideal score of 3.5. Treatment means were considered different when alpha was smaller than 5% and trends were considered significant when P-value varied from 0.05 to 0.10.

The hairball feeding trial was performed as a replicated switch-back design. The row factor was considered the diets and the column factor was the cats. The square, period, and cat nested within square were considered as random factors for the statistical analysis. Data were analyzed using the general linear model procedure for the mixed models (GLMMIX procedure in SAS; v. 9.4). Treatment means were considered different when alpha was smaller than 5% and trends were considered significant when P-value varied from 0.05 to 0.10.

Results and Discussion

Digestibility Study

Nutrient compositions of diets were similar and small differences in concentration of crude protein, crude fat, gross energy, and ash were related to differences in the composition of the test fiber sources (Table 6.1). Crude fiber content was included in the report because of its requirement by the American Association of Feed Control Officials (AAFCO, 2015) as part of the guaranteed analysis on pet food labels. Despite this requirement, crude fiber analysis only accounts for a portion of the insoluble fiber of the diet, since all the soluble components and some of the insoluble components of the dietary fiber are solubilized and removed from the sample by the acid and alkaline digestions (AOCS Ba 6a-05). In addition to the crude fiber content, total dietary fiber was reported. In this case, the diets containing CED and MGD had higher total dietary fiber content than BPD. These results were expected, as the total fiber content of CED and MGD were higher than BPD (102.62%, 90.00%, and 62.36% total dietary fiber respectively for CED, MGD, and BPD; Table 6.3).

The fiber profile of these experimental ingredients can be divided into two main categories: insoluble (like CED and MGD) and moderately soluble (like BPD) fiber sources. The high concentrations of insoluble fibers in CED and MGD are directly related to the composition

of the raw materials used for the production of these two ingredients. Cellulose is derived from the paper and pulp industry. During production, wood chips are digested and all the cellular content from the trees are removed with the exception of the cellulose (Dahl, 1884). Conversely, the raw materials for the production of Miscanthus grass only go through a grinding step. Thus, since no purification step occurs, the concentration of other plant components is much higher in Miscanthus grass than in cellulose. This results in higher concentrations of lignin (13.68% vs. 0.73%, respectively for Miscanthus grass and cellulose). Finally, beet pulp is the material remaining after sugar extraction from sugar beets. In this case, the soluble fiber content of beet pulp is about 3.5 times higher than Miscanthus grass and 10 times higher than cellulose. In addition to the chemical characterization, bulk density and particle size analysis were performed on the fiber sources. Cellulose was the least dense fiber source and had the smallest average particle size compared to the other tested fibers. Beet pulp was the opposite; wherein it was denser and had bigger particles on average compared to cellulose. Miscanthus grass was intermediate to these two standard fiber sources; however, it was numerically closer to cellulose values.

Cats maintained weight and body condition score throughout the duration of the experiment. Food intake was similar among treatments (average 76.1 g per day per cat; Table 6.4). While defecation frequency was similar among dietary treatments (average 1.19 defecations per day per cat), a difference in fecal consistency was observed. Cats fed BPD had softer stools than cats fed MGD diet. Additionally, the fecal score for BP was lower than the ideal score of 3.5. Despite the similar wet fecal output among the dietary fiber sources, cats fed BPD had an increased fecal moisture when compared to the cats fed MGD and CED diets. This increase in fecal moisture may account for the increase in stool softness. Similarly, Fahey et al. (1990a)

reported a quadratic decrease in fecal dry matter when the beet pulp concentration in dog foods increased, although the authors did not report fecal scores in their study. While these results are not completely understood, the fiber source could be the main cause of this increased fecal moisture. As noted here, and in other studies (Fahey et al., 1990b; Lewis et al., 1994; Diez et al., 1998) the addition of insoluble fibers to the diet did not increase fecal moisture. Thus, one may deduce that the soluble component of the fiber was the main cause of the increased water excretion. While the fiber water binding capacity is not sufficient to prevent water absorption (Tungland and Meyer, 2002), when it is fermented it may shift the water dynamics in the colon. One possible mechanism in which this occurs is through a decrease in luminal content pH (Biagi et al., 2010; Felix et al., 2013) which may cause a chemical irritation of the epithelial cells. As a response to the decrease in pH, the colon may decrease water absorption or secrete water and bicarbonate to increase the pH and decrease the irritation. As a result, the fecal water content would increase with the shift in fermentation end products and the decrease in pH. Another possible explanation for the increase in fecal moisture is that the fermentation end products shift the osmotic balance between the large intestine and the lumen, causing the luminal contents to pull water into the large intestine. However, these theories are yet to be confirmed.

Nutrient utilization was estimated using two different methods: total fecal collection and titanium dioxide. When measured using total fecal collection, cats fed BPD had a higher dry matter, organic matter, gross energy, and total dietary fiber digestibility than cats fed other dietary treatments ($P < 0.05$; Table 6.5). In contrast, cats fed CED had a higher crude protein digestibility than cats fed BPD (86.1% vs. 84.2%, respectively), and crude protein digestibility than cats fed MGD ($P = 0.0567$). Crude fat digestibility was lower for cats fed MGD than cats fed the other two treatments ($P < 0.05$, Table 6.5). When using titanium dioxide as an external

marker to estimate apparent total tract digestibility, a similar ranking of nutrient digestibility was reported for each diet (Table 6.5). Generally, BPD was more digestible when considering dry matter, organic matter, gross energy, and total dietary fiber. Cats fed CED and MGD had higher crude protein digestibility than cats fed BPD. The MGD had a lower crude fat digestibility when compared to the other two dietary treatments. Interestingly, the total dietary fiber digestibility of CED estimated by titanium dioxide was negative. While this may not be possible in theory, it is a good indication of the low utilization of this fiber source by the colonic microorganisms. Sunvold et al. (1995ab) evaluated cellulose as a fiber source in an in vitro fermentation model using different fecal inoculums (dog, cat, horse, human, pigs, and ruminal fluid) and incubation times (0, 6, 12, 24, and 48 h) and in all cases, cellulose was poorly fermented and generated very small amounts of fermentation end products compared to other more fermentable fiber sources. This further supports the theory that this fiber is not a readily fermented substrate.

The higher dry matter, organic matter, gross energy and total dietary fiber digestibility of BPD can be attributed to its fiber composition, because the soluble fiber is better utilized by the bacteria in the colon and fermented to various end products (lactate, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, H_2, CO_2, methane). This similar to the relationship reported by Sunvold et al., 1995ab; Cutrignelli et al., 2009. In these cases, the undigested and unabsorbed digesta enter the colon and are fermented, the organic matter is transformed into fermentation end-products and microbial mass. As a result, less material is excreted by the cats and an increase in digestibility is observed. Additionally, more microbial protein and fats are eliminated through the feces, thereby resulting in a decrease in crude protein and crude fat digestibility. In this study, crude protein digestibility was lower for cats fed BPD. However, the crude fat digestibility was not affected by dietary beet pulp. Similar to this work, Muir et al.

(1996) did not report a fiber effect due to the addition of cellulose or beet pulp in crude fat digestibility by dogs.

Hairball Study

Ingredient compositions of experimental diets were similar to the diets from the digestibility experiment, with the exception of a higher inclusion of flavor enhancer, and the use of rice flour as a replacement for Miscanthus grass in the control diet (Table 6.6). In this case, flavor enhancer was added at a higher level to avoid food refusals; however, one cat had to be removed from the experiment since it would not eat the food. Cats are known to be finicky and this was the first experience this colony of cats had with foods other than standard house diets. Nutrient compositions of both diets were also similar and small differences in crude protein, crude fat, and ash were likely due to the differences in composition between Miscanthus grass and rice flour (Table 6.7). As expected, the crude fiber and total dietary fiber content of MGH were higher than the COH.

Cat body weight was maintained throughout the duration of the experiment (Table 6.8). Food intake and defecation frequency were similar between treatments. However, cats fed MGH had harder stools than cats fed COH ($P < 0.05$). When comparing the fecal scores with the ideal score (3.5), cats fed COH had fecal consistency similar to the ideal score ($P = 0.6616$), but cats fed MGH had harder feces than the ideal (3.99 vs. 3.5, $P < 0.05$; Table 6.8). Wet fecal output was higher for cats fed MGH than cats fed COH ($P < 0.05$); however, no changes in fecal dry matter concentration were reported. The greater fecal output was expected, since the fiber content of MGH was much greater than that of COH (Table 6.7). As noted previously (Table 6.3), Miscanthus grass is a good source of insoluble fiber, making it a poorly fermentable fiber source. Because of this, the fiber may be eliminated in the feces, increasing fecal weight and volume.

It is important to understand how hairballs are formed in cats. Hairball formation is common among other animals beyond cats. Hairballs have been reported in dogs (Cannon, 2013), rabbits (Gillet et al., 1983), horses (Turner, 1986), beef calves (Abutarbush et al., 2004), and feral animals (lemur, Janssen et al., 1979; Canadian lynx, Kottwitz and Munsterman, 2013; cougar, Langohr et al., 2006). Despite reports of occurrence in other species, it is most concerning in cats in our homes. In addition, there is a difference in hairball regurgitation incidence when the length of the cat's hair differs, wherein long-haired cats were twice as likely to vomit a hairball than short-haired cats (Cannon, 2013). However, these results are based on cat owner surveys. Since this data was based on home use rather than controlled scientific and balanced studies, more information is still needed to better understand the relationship between hair type and hairball elimination.

The formation of hairballs in the stomach have been thought to be a combination of three factors: behavior, anatomy, and physiology. According to Panaman (1981) the cat spends about 25% of their awake time grooming themselves or other cats. This considerable amount of time contributes to hair ingestion by the animal. In addition, the cat's filiform papillae has several hook-like barbs facing the back of the oral cavity (Cannon, 2013). As a result, the conformation of the papillae hampers the removal of the collected hair by any method other than ingestion. Lastly, cats have a different fasting gastrointestinal contraction type than most other mammals called the migrating spike complex (MSC; De Vos, 1993). These fasting contraction patterns are considered important to move any type of undigested material to the large intestine. In the migrating motor complex contractions (MMC) start in the stomach and move toward the end of the small intestine (Wyse et al., 2003). Alternatively, the MSC starts at the duodenum and moves to the ileum (Bebchuk, 2002). Because there are no fasting contractions in the stomach (De Vos,

1993), the hair accumulates. In most cases, these hairballs are small and pass to the duodenum without complications; however, in some cases, they can be regurgitated or cause blockages in the gastrointestinal tract (Barrs et al., 1999).

In an attempt to increase the passage rate and stimulate gastric contraction, fibers have been added to cat foods to aid in passing the hairballs to the duodenum (Dann et al., 2004; Davenport et al., 2008, Patent no. 7,425,343 B2; Loureiro et al., 2017). For example, Dann et al. (2004) reported a decrease in clinical signs (vomiting, retching, and coughing) of hairballs when cats with a history of hairballs were supplemented with a 2 g gelatin capsule containing a blend of Psyllium husk and slippery elm (b.i.d) when compared to the control treatment with no added fiber. Conversely, Loureiro et al. (2017) reported no effects due to the beet pulp content (8% vs. 16%) of extruded cat foods in number of small, medium, large, or total hairballs, although there was a tendency for total number of hairballs relative to mass of fresh feces to increase. In addition, it is important to highlight that Loureiro et al. (2017) did not specify if the cats in this experiment were prone to hairballs (shorthaired cats). Finally, Armbrust et al. (2003) reported no effect of fiber content in extruded cat food on transit time; however, the authors found that round kibbles increased passage rate compared to triangular kibbles. These variable results show that there is not a consensus regarding whether fiber addition or supplementation is effective for the management of hairballs. Furthermore, there is a potential benefit of increasing gastrointestinal motility. Lewis and Heaton (1999) reported that the supplementation of (human) foods with plastic flakes increased passage rate by 24% compared to no supplementation. Thus, the use of an insoluble dietary amendment such as Miscanthus grass, may potentially have the same effects in cats as humans. Similar to the plastic flakes, insoluble fiber particles may stimulate the digestive tract contractions and increase digesta passage rate. As the passage rate increases, it is

expected that the hairballs will be smaller and move to the duodenum sooner. As a result, regurgitations and (or) intestinal blockages may be less likely to occur.

In the present study, there was only one hairball regurgitated; therefore, it was difficult to draw conclusions regarding the effects of the diet on the regurgitation of hairballs per se. These were also shorthaired cats not previously reported to be prone to hairballs. That creates a greater hurdle to evaluate the diet effects. As a result, the responses evaluated were the hair clumps in the feces. Hair clump count, hair clump count per cat per day, hair clump size, and total hair clump weight were similar between treatments ($P > 0.05$; Table 6.8). These results corroborate with the initial observation that hair turnover was similar between the two experimental periods and treatments. Additionally, this adds confidence that these results were due to the dietary treatments, rather than random variation between experimental periods or animals used.

Despite the lack of differences in fecal hair clump traits, hair retained in the strainer tended to be higher for cats fed MGH compared to COH. This result, in addition to the similar hair turnover between treatments, could be an indication that the addition of insoluble fibers to the diet improves motility and may aid in breaking down or prevent formation of the hair clumps. Subsequently, more hair was mixed with the fecal matter, and smaller hair masses were excreted. However, it is important to highlight that no differences were reported in the average hair clump size and count in this study. Total fecal hair weight tended to be lower for cats fed COH. In this case, when considering the amount of hair in the feces in relation to the fecal output, cats fed MGH had a lower total hair weight per gram dry fecal weight and less hair clumps per gram of dry fecal weight ($P < 0.05$; Table 6.8). While this result was unexpected, it may be an indication that more digesta passing through the gastrointestinal tract could aid in

more regular movement of the hairballs to the duodenum, in addition to possibly keeping the hairballs and hair clumps smaller due to the increase in motility.

Conclusion

The addition of insoluble fiber sources to cat foods may decrease the energy intake and digestibility, thus aiding in weight management. However, there may be an increase in fecal output as a result. Miscanthus grass had similar digestibility coefficients to cellulose, indicating that Miscanthus grass could be a viable alternative to cellulose in cat foods. Furthermore, the addition of Miscanthus grass to cat foods with the purpose of hairball management decreased total hair weight and hair masses per gram of dry feces. This provides a rationale for additional work to determine if greater particle size for Miscanthus grass in long-hair cats prone to hairballs may provide benefits.

Author Contributions to the Chapter

RAD: experiment conduction, data and sample collection, sample analysis, statistical analysis, data interpretation, and manuscript preparation.

CGA: experiment design, data interpretation, and manuscript revision.

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Chapter 6 Tables

Table 6.1 Ingredient composition of experimental diets.

Ingredient	Percentage
Fiber source	10.00
Ration	84.34
Chicken byproduct meal low ash	35.22
Brewers rice	14.07
Corn	14.07
Wheat	14.07
Corn gluten meal (75%CP)	5.00
Salt	0.40
Potassium Chloride	0.26
Choline Chloride (60%)	0.20
Calcium Carbonate	0.20
Vitamin Premix [1]	0.20
Trace Mineral Premix [2]	0.20
Fish Oil	0.10
Taurine	0.10
Natural Antioxidant	0.10
Titanium oxide	0.40
Chromium sesquioxide	0.25
Chicken fat*	4.01
Flavor enhancer*	1.00

* Included as coating after diet were dried to less than 10% moisture.

[1] Vitamin E Supplement (79,887 IU*kg^{-1}), Niacin Supplement (64,736 mg*kg^{-1}), Calcium Pantothenate (12,186 mg*kg^{-1}), Vitamin A Supplement (17,162,998 IU*kg^{-1}), Thiamin Mononitrate (14,252 mg*kg^{-1}), Pyridoxine Hydrochloride (5,537 mg*kg^{-1}), Riboflavin Supplement (4,719 mg*kg^{-1}), Vitamin D3 Supplement (920,000 IU*kg^{-1}), Biotin (70 mg*kg^{-1}), Vitamin B12 Supplement (22 mg*kg^{-1}), Folic Acid (720 mg*kg^{-1}).

[2] Zinc Sulfate (88,000 mg*kg^{-1}), Ferrous Sulfate (38,910 mg*kg^{-1}), Copper Sulfate (11,234 mg*kg^{-1}), Manganous Oxide (5,842 mg*kg^{-1}), Sodium Selenite (310 mg*kg^{-1}), Calcium Iodate (1,584 mg*kg^{-1}).

* Added during the coating to the dried kibbles.

Table 6.2 Nutrient composition of experimental diets expressed on a dry matter basis.

Composition	MGD[1]	CED[1]	BPD[1]
Dry matter, %	94.53	94.48	94.60
Crude protein, %	35.40	34.20	33.80
Crude fat, %	11.40	12.00	11.60
Ash, %	7.16	7.01	7.00
Crude fiber, %	5.56	8.90	2.95
Total dietary fiber, %	13.76	14.48	10.88
Gross energy[2], kcal*kg^{-1}	4,839	4,823	4,839

[1] Dietary treatments; MGD: Miscanthus grass, BPD: beet pulp, CED: cellulose.

[2] Analyzed by calorimetry (model 1351, Parr Instrument Company, Moline, IL).

Table 6.3 Chemical and physical characterization of experimental fiber sources used to produce cat foods for the digestibility and hairball feeding trials.

Fiber source	Miscanthus grass	Cellulose	Beet pulp
	Cehmical[1], %		
Dry matter	95.00	95.30	92.53
Crude fiber	47.58	76.29	20.21
Acid detergent fiber	56.53	84.58	26.26
Neutral detergent fiber	77.68	92.76	34.15
Acid detergent lignin	13.68	0.73	6.38
Total dietary fiber	90.00	102.62	62.36
Insoluble fiber	82.74	100.00	35.99
Soluble fiber	7.26	2.62	26.37
	Physical		
Bulk Density, $g*mL^{-1}$	0.31	0.19	0.73
DGW ± S_{gw}[2], µm	103.46 ± 76.39	77.33 ± 44.47	193.78 ± 194.83

[1] Expressed in dry matter basis.

[2] DGW: geometric mean diameter, S_{gw}: standard deviation, ASABE, 2008

Table 6.4 Food intake, defecation frequency, fecal score, wet fecal output, and fecal dry matter of cats fed diets with different fiber sources (N = 12).

Diet	MGD[1]	CED[1]	BPD[1]	SEM[2]	P-value
Body Weight, kg	4.59	4.60	4.61	0.42	0.9157
Body Condition Score	5.00	5.04	5.08	0.44	0.8695
Food Intake, g/d/cat	74.9	78.9	74.7	4.71	0.6353
Defecation Frequency, no/d*cat	1.25	1.25	1.07	0.098	0.1393
Fecal Score[3]	3.32[a]	3.21[ab]	2.84[b]	0.15	0.0439
Fecal Score[4] = 3.5, P-value	0.3356	0.1114	<0.0001		
Wet Fecal Output, g/d*cat	49.0	56.8	51.3	5.27	0.3050
Fecal Dry Matter, %	34.23[a]	33.62[a]	26.98[b]	1.23	0.0002

[ab] Means with unlike superscripts differ (P < 0.05).

[1] Dietary treatments; MGD: Miscanthus grass, BPD: beet pulp, CED: cellulose

[2] SEM: Standard error of the mean

[3] Fecal score: 1 – liquid diarrhea, 5 – dry hard pellets, 3.5 – ideal.

[4] t-test p-value from the comparison average fecal score = 3.5.

Table 6.5 Apparent total tract digestibility estimated by total fecal collection (TFC) and Titanium dioxide (TIO) of experimental diets enriched with fiber and fed to cats.

Digestibility, %	MGD[1]	CED[1]	BPD[1]	SEM[2]	P-value
		TFC			
Dry Matter	76.2[b]	75.5[b]	81.1[a]	0.89	<0.0001
Organic Matter	80.5[b]	79.4[b]	85.9[a]	0.74	<0.0001
Crude Protein	85.8[ab]	86.1[a]	84.2[b]	0.88	0.0567
Crude Fat	85.0[b]	89.6[a]	89.2[a]	0.73	<0.0001
Gross Energy	81.7[b]	80.9[b]	85.6[a]	0.72	<0.0001
Total Dietary Fiber	20.8[b]	12.2[c]	39.7[a]	3.03	<0.0001
		TIO			
Dry Matter	75.0[b]	73.0[c]	80.6[a]	0.51	<0.0001
Organic Matter	79.1[b]	77.1[c]	83.4[a]	0.48	<0.0001
Crude Protein	84.7[a]	85.1[a]	82.1[b]	0.94	0.0323
Crude Fat	83.8[b]	87.9[a]	87.1[a]	0.83	0.0020
Gross Energy	80.3[b]	78.8[b]	83.1[a]	0.64	0.0001
Total Dietary Fiber	15.2[b]	-2.6[c]	44.1[a]	1.47	<0.0001

[abc] Means with unlike superscripts differ ($P < 0.05$).

[1] Dietary treatments; MGD: Miscanthus grass, BPD: beet pulp, CED: cellulose

[2] SEM: Standard error of the mean.

Table 6.6 Ingredient composition of hairball experimental diets, expressed on an as is basis.

Ingredient, %	MGH[1]	COH[1]
Chicken by-product meal, low ash	35.22	35.22
Rice flour	14.07	24.07
Corn	14.07	14.07
Wheat	14.07	14.07
Miscanthus grass	10.00	--
Corn gluten meal (75% CP)	5.00	5.00
Salt	0.40	0.40
Potassium chloride	0.26	0.26
Choline chloride (60% dry)	0.20	0.20
Dicalcium phosphate	0.20	0.20
Calcium carbonate	0.20	0.20
Vitamin premix [2]	0.20	0.20
Trace mineral premix [3]	0.15	0.15
Fish oil	0.10	0.10
Taurine	0.10	0.10
Chicken fat*	4.00	4.00
Flavor enhancer*	1.50	1.50

* Included as coating after diet were dried to less than 10% moisture.

[1] Dietary treatments; MGH: Miscanthus grass, COH: control.

[2] Vitamin E Supplement (79,887 IU*kg^{-1}), Niacin Supplement (64,736 mg*kg^{-1}), Calcium Pantothenate (12,186 mg*kg^{-1}), Vitamin A Supplement (17,162,998 IU*kg^{-1}), Thiamin Mononitrate (14,252 mg*kg^{-1}), Pyridoxine Hydrochloride (5,537 mg*kg^{-1}), Riboflavin Supplement (4,719 mg*kg^{-1}), Vitamin D3 Supplement (920,000 IU*kg^{-1}), Biotin (70 mg*kg^{-1}), Vitamin B12 Supplement (22 mg*kg^{-1}), Folic Acid (720 mg*kg^{-1}).

[3] Zinc Sulfate (88,000 mg*kg^{-1}), Ferrous Sulfate (38,910 mg*kg^{-1}), Copper Sulfate (11,234 mg*kg^{-1}), Manganous Oxide (5,842 mg*kg^{-1}), Sodium Selenite (310 mg*kg^{-1}), Calcium Iodate (1,584 mg*kg^{-1}).

* Added during the coating to the dried kibbles.

Table 6.7 Hairball dietary treatments nutrient composition expressed on a dry matter basis.

Composition, %	MGH[1]	COH[1]
Dry matter	94.55	95.21
Crude protein	34.40	34.80
Crude fat	11.30	11.30
Ash	6.73	7.31
Crude fiber	5.51	0.43
Total dietary fiber	14.11	5.43
Metabolizable energy[2], kcal*kg-[1]	3,446	3,626

[1] Dietary treatments: MGH: Miscanthus grass, COH: Control.

[2] Metabolizable energy estimated using Atwater values (crude protein = 3.5, nitrogen-free extract = 3.5, crude fat = 8.5).

Table 6.8 Average body weight, food intake, defecation frequency, fecal score, wet fecal output, and fecal dry matter (mean ± standard error of the mean) of cats fed diets with different fiber content.

	MGH[1]	COH[1]	P-value
Body Weight, kg	4.97 ± 0.38	4.99 ± 0.38	0.5835
Food Intake, g/d/cat	40.24 ± 1.81	38.74 ± 1.76	0.3570
Defecation Frequency, no/d*cat	1.30 ± 0.12	1.22 ± 0.12	0.5521
Fecal Score[2]	3.99^{a} ± 0.27	3.36^{b} ± 0.26	0.0065
Fecal Score = 3.5, P-value	0.0093	0.6616	
Wet Fecal Output, g/d*cat	47.0^{a} ± 3.03	28.8^{b} ± 2.96	<0.0001
Fecal Dry Matter, %	48.9 ± 3.24	47.3 ± 3.15	0.6192
Hair clump count, no	13.19 ± 2.77	11.58 ± 2.74	0.3050
Hair clump count per day, no*d^{-1}	2.64 ± 0.55	2.32 ± 0.55	0.3050
Hair clump size[3]	1.79 ± 0.23	2.22 ± 0.22	0.1437
Hair clump weight, mg	36.8 ± 8.91	32.6 ± 8.73	0.6110
Total hair clump weight, mg	674 ± 213	420 ± 209	0.1167
Hair retained in strainer, mg	310 ± 81	248 ± 80	0.0884
Total hair weight, mg	1641 ± 214	1362 ± 210	0.0790
Total hair weight per dry fecal weight, mg*g^{-1}	14.59^{b} ± 2.92	21.04^{a} ± 2.90	0.0004
Hair clumps per dry fecal weight, no*g^{-1}	0.12^{b} ± 0.039	0.19^{a} ± 0.039	0.0013

[ab] Means with unlike superscripts differ (P < 0.05).

[1] Dietary treatments; MGH: Miscanthus grass (n=11), COH: control (n=12).

[2] Fecal score: 1 – liquid diarrhea, 5 – dry hard pellets, 3.5 – ideal.

[3] Hair mass size: extra small (<10.0 mm x <5.0 mm; length x diameter), small (10.0 - 20.0 mm x 3.5 - 6.5 mm), medium (20.0 - 30.0 mm x 4.0 - 7.0 mm), large (30.0 - 40.0 mm x 4.5 - 8.5 mm), and extra-large (>40.0 mm x >5.0 mm).

Chapter 7 - The effects of fiber source on organic matter disappearance and fermentation end-products by an in vitro dog fecal inoculum model

Abstract

Dietary fibers can influence a dog's overall health; however; high concentrations of soluble dietary fibers can cause soft stools. An *in vitro* model could be useful to predict the rate fibers are fermented once they reach the colon. The objectives of the study were to determine the effects of fiber source on organic matter disappearance (OMD) and fermentation end-product concentrations using an in vitro fermentation procedure and dog fecal inoculum. Miscanthus grass (MG), cellulose (CE), beet pulp (BP), pea fiber (PF), and sorghum bran (SB) were digested prior to the inoculation with buffered dog feces. Fecal samples were collected and maintained in anaerobic conditions until the dilution and inoculation. Test tubes containing the fibrous substrates were incubated for 0, 4, 8, and 12 h at 39ºC. Short chain fatty acids (SCFA), branched chain fatty acids (BCFA), and OMD were determined for each fiber source and time point. Beet pulp had the highest OMD and SCFA production of all tested fiber sources (38.6% OMD, 2.72 mmol*g^{-1} of substrate of SCFA). Sorghum bran had higher concentrations of BCFA (0.060 mmol*g^{-1} of substrate) and intermediate OMD compared to the other tested fibers. Cellulose and MG were poorly fermented with the lowest OMD, SCFA, and BCFA compared to other fibers. In conclusion MG could be used as an insoluble minimally fermentable replacement fiber for CE in dog foods.

Introduction

Various fiber sources are used in pet foods with different purposes, such as energy dilution, gut health, and hairball management (Castrillo et al., 2001; Loureiro et al., 2014; Floerchinger et al., 2015). For these purposes, select fibers and various inclusion levels were used. Fiber type (soluble vs. insoluble fibers) and the concentration of the fiber in the diet can impact nutrient utilization and stool quality (Fahey et al., 1990ab, Wichert et al., 2002). Cellulose and beet pulp are considered standard fiber sources in pet foods and have been extensively studied in dog models (Fahey et al., 1990ab; Sunvold et al., 1995ab; Wichert et al., 2002; Prola et al., 2010). However, pet food companies continue to explore alternative ingredients to sustain industry growth and consumer demand. Miscanthus grass, sorghum bran, and pea fiber could be such alternative fiber sources. However, little is known about the effects of these fiber sources on nutrient utilization, stool quality, and gut health.

Perhaps, their effects on stool quality and gut health could be evaluated using an *in vitro* model with canine fecal inoculum (Sunvold et al., 1995ab; de Godoy et al., 2015). Using this technique, the rate of production and the concentration of fermentation end-products can be estimated. Additionally, the results could be used to guide food formulators on the purpose of each fiber source (gut health vs. energy dilution) and their inclusion levels. However, results may be inconsistent, because there is variation in fiber composition depending on the conditions that the crops were grown (Fahey et al., 1990b) and processed (Montagne et al., 2003), in addition to variation between laboratory evaluation (Barry et al., 1990). Regardless, characterization of fermentability could be beneficial for pet food formulation and pet's health. The objective of this study was to determine the fermentation end-products for different fiber sources using an *in vitro* model with dog fecal inoculum.

Materials and Methods

Fiber Sources Preparation and Characterization

Fiber sources were Miscanthus grass (MG, Renew Biomass, Springfield, MO), cellulose (CE), beet pulp (BP), and pea fiber (PF, Fairview Mills, Seneca, KS), and sorghum bran (SB, Hall Ross Flour Mill, Kansas State University; Alvarenga et al., 2018). These fibers were selected because they have been evaluated in animal feeding studies in the Pet Food Processing Lab at Kansas State University (SB, Alvarenga et al., 2018; PF, Pontious et al., 2018; MG, CE, BP, Donadelli and Aldrich, 2019; Table 7.1). Prior to the incubation, samples were pre-digested with α-amylase, protease, and amyloglucosidase (total dietary fiber assay kit, Sigma-Aldrich, catalog no. TDF100A-1KT) simulating the digestion in the small intestine of the dog. Briefly, 10 g of sample was mixed with 0.08M phosphate buffer (pH = 6) and the α-amylase. The samples were placed in a water bath at 95ºC and were digested for 15 min once the sample reached 95ºC. Next, the pH was adjusted to 7.5 with sodium hydroxide (0.275 N) after the samples cooled to room temperature and protease was added to the samples. Next samples were digested for 30 min once they reached 60ºC in a water bath. Then sample pH was adjusted to 4.3 with hydrochloric acid (0.325 N) once the samples cooled to room temperature and amyloglucosidase was added. Next, samples were digested for 30 min once they reached 60ºC in a water bath. After the samples were cooled to room temperature, four volumes of 95% ethanol were added. Samples were allowed to sit on the bench-top overnight. On the following day, the samples were filtered, then the sample was rinsed with two 100 mL volumes of 95% ethanol and two 100 mL volumes of acetone. After the filtration, the samples were oven dried overnight at 55ºC. On the following day samples were ground to pass a 1-mm screen (Retsch ZM200, Germany). Protein content (AOAC 990.03) was measured on the substrates after the simulated small intestinal digestion, total dietary fiber (TDF;

Prosky et al., 1985), and insoluble fiber (Prosky et al., 1988) were determined on the fiber samples, and TDF and insoluble fiber were determined on the lab food provided to the animals. Soluble fiber was calculated for the fiber samples and food by subtracting insoluble fiber from the TDF content of each sample. Additionally, crude protein (CP; AOAC 990.03) was determined on the fiber sources after the simulated digestion. Fat by acid hydrolysis (AOAC 954.02), ash (AOAC 942.05), and crude fiber (AOCS Ba6a-05) were determined on the lab food.

Dog Donors and Inoculum Preparation

Beagle dog donors were group housed in the Large Animal Research Center of Kansas State University. Two dogs were grouped per pen with access to outside fenced exercise area. The laboratory diet (Table 7.2) was provided twice daily for each dog according to their energy requirements for at least 2 weeks prior to the fecal sample collection. The laboratory diet was analyzed for its moisture, CP, fat by acid hydrolysis, ash, crude fiber, TDF, insoluble, and soluble fiber composition as previously described. Prior to the incubation of the fiber samples, feces were collected fresh within 15 min after defecation. Four dogs (2 neutered males and 2 spayed females) defecated within a 15 min span and their feces were collected for the preparation of the inoculum. Each fecal sample was stored in a plastic bag and the air was removed from the bag to decrease contamination with O_2. Next the bags were placed in an insulated container which was warmed to 37ºC. The fecal samples were transported to the lab and 25 g ± 0.1g from each feces was pooled to produce the inoculum.

Next, the pooled fecal sample was mixed with 1 L of anaerobic dilution solution (1:10 feces:dilution solution, wt:vol; Table 7.2) under copper scrubbed CO_2. Once completely mixed, the solution was filtered through 4 layers of cheese cloth under copper scrubbed CO_2. The solution was kept at 39ºC until inoculation of sample tubes.

Incubation Preparation and Organic Matter Disappearance Determination

Fiber samples were weighed (310 mg ± 0.1 mg, in triplicate) in 50 mL centrifuge tubes for each one of the 4 time points (0, 4, 8, and 12 h). To each tube, 26 mL of media solution (Table 7.2) was added. Next, each tube was flushed with copper scrubbed CO_2 and closed with a rubber stopper equipped with a one-way valve. Tubes were then placed in the refrigerator overnight to allow hydration of the fibers. On the following day, the samples were placed in the water bath at 39ºC for 1 h prior to the inoculation. In addition to the tubes with the fiber samples, four tubes for each time point were filled with media solution to be used as blanks. We decided to use 4 tubes for the blanks instead of 3 tubes based on previous experiments that showed a greater variation in the blanks.

Tubes were inoculated with 4 mL of inoculum (filtered anaerobic dilution solution) starting tubes from time 12 h, then 8 h, 4 h, and lastly 0 h. This approach was used to decrease the time that the tubes from time 0 h would be fermented until all tubes were inoculated. After inoculation, tubes were flushed with copper scrubbed CO_2, closed with a rubber stopper equipped with a one-way valve, and incubated in water bath at 39ºC for the pre-determined time points. After each incubation time, two 1 mL sub-samples from each tube were transferred to microcentrifuge tubes for fermentation end-products determination. The remaining liquid and solid residue in the centrifuge tube was transferred to a beaker, mixed with 112 mL of 95% ethanol, and allowed to rest overnight. On the following day, the samples were filtered using a dried pre-weighed ashless Whatman filter paper (catalog no. 1541-110) and rinsed with two 10 mL volumes of 95% ethanol and two 10 mL volumes of acetone. Next, residues and filter were dried in a convection oven overnight at 105ºC. The dry weight of the filter and residue was recorded the following day. Organic matter disappearance (OMD) was calculated as follows:

$$OMD = 1 - \frac{OM\ residue - OM\ blank}{Initial\ OM}$$

wherein *OM residue* is the organic matter on the sample after the incubation and filtration in g, *OM blank* is the organic matter on the blank after incubation and filtration in g, and *Initial OM* is the initial organic matter in the sample prior to incubation in g.

Fermentation End Produces Determination

As reported previously, 1 mL was transferred from each centrifuge tube in duplicate to microcentrifuge tubes. Next, 250 µL of 25% m-phosphoric acid was added to each microtube. The micro tubes were then stored in the freezer (-17ºC) for 24 h prior to analysis. Next, the microtubes were thawed and centrifuged (15,000 g for 15 min). Then, an aliquot of 0.4 mL from the clear supernatant was transferred to a GC vial containing 20 µL of 6N sodium hydroxide and 1.2 mL of pivalic internal standard. The samples were analyzed using a 2 m x 2 mm Carbopack B-DA (Supelco) column. The injection port temperature was 200ºC, the oven temperature was 175ºC, and the flame ionization detector temperature was 200ºC. Flow rate was set at 24 mL*min^{-1} with nitrogen gas as a carrier. The final concentration for each fermentation end-product was corrected for the sample weight and blank values for their respective time points.

Statistical Analysis

The experiment was performed as a completely randomized design, with 50 mL centrifuge tube being the experimental unit. Data were analyzed using the general linear model procedure (SAS, v. 9.4). In addition, a t-test was performed to assess if the fermentation end-products concentration for the different fiber sources and time points were different than zero. Differences were considered significant if P was smaller than 0.05, trends were considered when P ranged from 0.05 and 0.10.

Results and Discussion

Fiber composition was variable with respect to the insoluble, soluble, and TDF (Table 7.1). Crude protein content on the CE substrate was higher than concentrations reported by Sunvold et al. (1995ab) and de Godoy et al. (2015). However, the TDF content was within the range from reports in the literature (Sunvold et al., 1995ab; de Godoy et al., 2015). Crude protein content of BP substrate was higher than values reported by Fahey et al. (1990ab) and Bosch et al. (2008); conversely, the TDF value was lower than reports by Sunvold et al. (1995ab) and de Godoy et al. (2015). Pea fiber substrate CP was lower compared to report by Bosch et al. (2008). Sorghum bran TDF content was similar to that reported by Alvarenga et al. (2018), although the protein content was higher. These differences in composition of the ingredients are known to occur when comparing agricultural by-products. This variation was noted by other authors and it could be due to differences in the conditions that these crops were produced (Fahey et al., 1990b) and differences in processing conditions to generate such products (Montagne et al., 2003). In addition, Barry et al. (1995) reported that there is variation in TDF analysis among different laboratories analyzing the same fiber source using the same method. The test fibers can be categorized by their CP, insoluble and soluble fibers content. In this case, Cellulose and MG have a high content of insoluble fiber and low content of CP and soluble fiber. Beet pulp has a higher concentration of soluble fiber compared to the other test fiber sources. Finally, PF and SB have intermediate concentrations if insoluble and soluble fibers, but SB has a much higher CP content than PF.

The results and discussion for OMD, SCFA and BCFA concentration presented here will be based on the 12 h time point unless otherwise specified (Table 7.3). Organic matter disappearance was greater for BP compared to other tested fibers regardless of the time point ($P < 0.05$). After 12 h of incubation, MG and CE had the lowest OMD compared to the other fiber

sources and PF and SB had intermediate values (1.3%, 2.6%, 6.9%, and 10.6%, respectively). The negative OMD value for MG on time 8 h (-0.8%), while unlikely, could be an error associated with the inoculum. It was not possible to separate all the fecal particles when filtering with the cheese cloth (i.e. the solution had a brown color after the filtration); therefore, some residual material from the inoculum might account for this result. The OMD of BP was lower when compared to values reported by Sunvold et al. (1995ab); however, CE OMD was similar. This could be a result of the lower TDF content of the BP used in this experiment compared to the one used by Sunvold et al. (1995a; 51.86% vs. 68.4%, respectively); therefore, with less organic matter fermented and the OMD would be lower. Sorghum bran in this experiment had similar TDF content to the rice bran evaluated by Sunvold et al. (1995a), although the OMD for rice bran was higher than OMD for SB (34.8% vs. 10.6% respectively). While the TDF content of these fiber sources was similar, the soluble fiber content of rice bran likely is much higher than the SB. Pea fiber maximum rate of gas production was reported to be about half of the rate of BP by Bosch et al. (2015); however, the OMD of PF about six times lower than BP OMD (38.6% vs. 6.9%, respectively for BP and PF). While the fermentation rate may be faster for BP, the OMD values agree with the soluble fiber content in these fiber sources, wherein BP has about 4 times more soluble fibers than PF (Table 7.1). As noted previously, the MG and CE OMD were similar, which could be due to their high insoluble and low soluble fiber composition; thereby, less material was available for the microorganisms to ferment, resulting in lower OMD compared to the other tested fibers (Table 7.3).

Acetate concentration was highest for BP, followed by PF and SB, and lowest for MG and CE ($P > 0.05$, Table 7.3). In the animal acetate is absorbed and transported by the blood stream to various peripheral organs. In these organs, acetate can be used as fuel source (e.g. muscle) or be

deposited as fat (e.g. adipose tissue; Bergman, 1990). Most of propionate is converted to glucose in the liver (Bergman, 1990). For our work propionate concentration increased over time for all fiber sources. Additionally, BP had the highest concentration (0.558 mmol*g^{-1} of substrate), SB had the second highest production (0.227 mmol*g^{-1} of substrate), followed by PF (0.127 mmol*g^{-1} of substrate), and MG and CE had the lowest values (0.023 and 0.018 mmol*g^{-1} of substrate, respectively).

Butyrate is perhaps the most important fermentation end-product for animal health. This SCFA promotes health benefits such as prevention of colonic cancer (McIntyre et al., 1993; Wong et al., 2005; Comalada et al., 2006) and chronic inflammation (Roediger, 1990, Vernia et al., 2003, Hamer et al., 2008), promotion of satiety (Delzenne et al., 2005; Karaki et al., 2007), improvement of defense barriers in the colon (Deplancke and Gaskins, 2001; Gaudler et al., 2004), and decreases in oxidative stress (Rosignoli et al., 2001; Toden et al., 2007). Butyrate is the preferred fuel source for colonocytes (Velazquez et al., 1997; Hamer et al., 2008). In this study, butyrate concentration was highest for BP and lowest for CE (0.105 vs. -0.003 mmol*g^{-1} of substrate, respectively). Similar results were also reported by Sunvold et al. (1995ab) and de Godoy et al. (2015). Sorghum bran led to the second highest butyrate concentration, followed by PF, and then MG ($P < 0.05$; Table 7.3). The butyrate concentration at 12 h of incubation was lower for the SB compared to rice bran (Sunvold et al., 1995a; 0.051 mmol* g^{-1} of substrate vs. 0.26mmol*g^{-1} of organic matter), thus rice bran might be considered a better fiber source for pet foods aiming gut health claims Butyrate concentration was higher for MG than CE ($P < 0.05$). This result could be related to how these ingredients are produced. Cellulose is a purified ingredient made from wood chips, in which most of the soluble fibers and lignin has been removed from the raw materials (Dahl, 1884). Differently, MG is produced from the ground dried canes of *Miscanthus giganteus* without any

purification steps. Therefore, the higher concentrations of other constituents than cellulose from the plant cell wall are not removed from the final ingredient which allowed them to be fermented and result in a higher concentration of butyrate and isovalerate (Table 7.3). Short chain fatty acid production was higher for BP, then SB and PF, and MG and CE produced the lowest concentration of SCFA. Similar results were reported by Barry et al. (1995), wherein the production of SCFA of sugar beet fiber was higher than that of CE (25.7 mmol*L^{-1} vs. 1.7 mmol*L^{-1}, respectively). In addition, these authors reported a high variation when comparing results from different laboratories. For example, sugar beet fiber SCFA content varied from 6.0 to 53.7 mmol*L^{-1}.

In addition to the SCFA, minor and BCFA were determined (Table 7.3). For the sake of this discussion, valerate will be grouped with the BCFA because its concentration was much lower than the other measured fermentation end products; however, it is important to state that valerate is a straight chain fatty acid, not branched. Unlike the SCFA, branched volatile fatty acids are exclusively produced from amino acids rather than a carbohydrate and protein sources (Bergman, 1990; Topping and Clifton, 2001; Blacheir et al., 2007). This may provide some explanation for their much smaller concentrations compared to SCFA (Table 7.3). In general, their concentration has been reported to be about 5 to 10 % of SCFA (Middelbos et al., 2007; Nery et al., 2012); however, the proportion can change if protein is used as substrate in an *in vitro* model (Urrego et al., 2017). Isobutyrate and isovalerate are produced from valine and leucine, respectively (Balchier et al., 2007). Possibly isobutyrate is the BCFA with the most importance, because of its similarities with butyrate (Dagher et al., 1996; Charney et al., 1999). Isobutyrate concentration was similar among fiber sources after 12 h of incubation. However, isovalerate concentration was higher for SB, followed by BP and MG, and CE and PF had the lowest contents ($P < 0.05$; Table 7.3). Valerate concentration was low among all treatments. Although, SB had the highest concentration (0.0031

mmol*g^{-1} of substrate) which was about six times higher than MG (the second highest; 0.0005 mmol*g^{-1} of substrate). Valerate concentration was similar among MG, CE, BP, and PF after 12 h of fermentation (Table 7.3). Valerate concentration for PF was negative (Table 7.3). Miscanthus grass and CE had several fermentation end-product concentrations that were not different than zero for 4 and 8 h of fermentation. Pea fiber had similar results for the BCFA and valerate concentrations ($P > 0.05$; Table 7.3). These results indicate that these fiber sources were resistant to microbial degradation and no net production of SCFA and BCFA were recorded until 8 h of fermentation. Therefore, these substrates needed more time to be utilized and even after 12 h of incubation small concentrations of fermentation end products were produced. Branched chain fatty acids concentrations were higher for SB than all other tested fiber sources ($P < 0.05$; Table 7.3). Finally, total volatile fatty acid production was higher for BP than PF and SB, with the lowest concentrations for MG and CE (2.74, 0.66, 0.44, 0.13, and 0.06 mmol*g^{-1} of substrate, respectively; Table 7.3).

The concentration of SCFA and BCFA is directly related to the content of soluble fibers and amino acid concentrations, respectively, but not necessarily the crude protein content. For example, BP had the highest content of soluble fibers compared to the other test fibers (Table 7.1), and yielded the highest production of SCFA after 4, 8, and 12 h of incubation (Table 7.3). Similarly, when considering the protein content of the substrates, SB had a higher content of CP (Table 7.1) and produced the highest concentration of BCFA. Beet pulp had a higher CP concentration than PF, but the BCFA concentration after 12 h of incubation was similar to PF. In addition, MG with about 3.5 times less CP than BP produced similar concentration of BCFA to BP. These results might be explained by the amino acid composition of each fiber source. As noted previously, the fermentation of valine and leucine will produce isobutyrate and isovalerate,

respectively (Balchier et al., 2007). Thus, BP should have low concentration of these two amino acids compared to MG and PF; which would explain the lower production of BCFA by BP.

In addition to the volatile fatty acid concentrations, their relative proportions in relation to the total was evaluated (Table 7.4). Similar to the concentration data, the discussion will be focused on the 12 h incubation values. In proportion to the total volatile fatty acids production, Miscanthus grass fermentation yielded a higher proportion of acetate, butyrate, and isovalerate compared to CE (Table 7.4). In addition, BP fermentation resulted in a low proportion of propionate (20.4%) compared to CE and SB (31.2 and 29.3, respectively). However, the proportion of SCFA was similar among the tested fiber sources after 12h of incubation (Table 7.4). Pea fiber fermentation resulted in lower proportions of isovalerate and valerate compared to MG, SB, and CE (Table 7.4). Finally, SB had high proportions of propionate and butyrate (29.3 and 6.6%, respectively; Table 7.4).

While the proportions of the volatile fatty acids may change depending on the fiber sources (Barry et al., 1995; Sunvold et al., 1995ab, de Godoy et al., 2015), there is no known competition for the absorption among the different fermentation end-products since the majority is transported by passive diffusion through the cell membrane (Bergman, 1990; Topping and Clifton, 2001). The important aspect of the fermentation is the rate of production of these products. As fermentation intensifies, there is an accumulation of flatus in the large intestine (Yamka et al., 2006) which could cause discomfort. Additionally, the increase in concentration of fermentation end-products could shift the osmotic balance in the colon and favor water and sodium transport towards the lumen. Thus, feeding a diet rich in soluble and rapidly fermentable fiber could lead to flatulence and diarrhea. Therefore, the fiber source composition included in the diet should be considered to prevent these side effects.

Conclusion

Fermentation end-products content increased as the soluble fiber content of the substrate increased. Similarly, as more protein was present in the substrate, more isobutyrate and isovalerate were produced. Beet pulp generated the highest concentrations of the individual and overall total short chain fatty acids. Pea fiber and SB were intermediate in the production of SCFA; however, SB had the highest production of valerate and isovalerate. Production of all SCFA and BCFA was lower when MG and CE were used as substrates.

Author Contributions to the Chapter

RAD: experiment conduction, data and sample collection, sample analysis, statistical analysis, data interpretation, and manuscript preparation.

CGA: experiment design, data interpretation, and manuscript revision.

ET: data interpretation, and manuscript revision.

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Chapter 7 Tables

Table 7.1 Laboratory dog food and individual test fiber composition (dry matter basis).

Composition, %	Food	MG[1]	CE[1]	BP[1]	PF[1]	SB[1]
Dry matter	92.79	95.39	96.30	94.33	93.46	93.86
Crude protein*	30.20	2.89	1.70	11.16	3.49	20.77
Fat	18.20	nd	nd	nd	nd	nd
Ash	7.75	nd	nd	nd	nd	nd
Crude fiber	2.25	nd	nd	nd	nd	nd
TDF[2]	9.85	80.92	94.91	51.86	71.93	22.90
Insoluble fiber	8.94	80.86	92.89	30.62	65.07	18.50
Soluble fiber[3]	0.91	0.06	2.02	21.24	6.86	4.40

[1] Fibrous substrate: MG: Miscanthus grass, CE: cellulose, BP: beet pulp, PF: pea fiber, SB: sorghum bran.

[2] TDF: total dietary fiber.

[3] Calculated, Soluble fiber = TDF – Insoluble fiber.

nd: not determined.

* Analyzed on the substrates after simulated small intestinal digestion.

Table 7.2 Composition of inoculation medium and anaerobic dilution solutions.

Solution	Medium	Anaerobic Dilution
Solution A[1], mL	330.0	37.50
Solution B[2], mL	330.0	37.50
Mineral Solution[3], mL	10.00	-
Vitamin Solution[4], mL	20.00	-
Folate-biotin solution[5], mL	5.00	-
Riboflavin solution[6], mL	5.00	-
Hemin solution[7], mL	2.50	-
Resazurin solution[8], mL	1.00	1.00
Water, mL	296.0	854.0
Yeast extract, g	0.50	-
Trypticase, g	0.50	-
Na_2CO_3, g	4.00	6.37
Cysteine hydrochloride, g	0.50	0.50

[1] Solution A - 5.4 g sodium chloride, 5.4 g ammonium sulfate, 2.7 g potassium phosphate monobasic anhydrous, 0.18 g calcium chloride dihydrate, 0.12 g magnesium chloride hexahydrate, 0.06g manganese chloride tetrahydrate, 0.06g cobalt chloride hexahydrate, to 1 L with distilled water.

[2] Solution B - 2.7 g potassium phosphate dibasic anhydrous to 1 L with distilled water.

[3] Mineral Solution - 500 mg of ethylenediaminetetraacetic acid, 200 mg iron (II) sulfate heptahydrate, 30 mg m-phosphoric acid, 20 mg cobalt chloride hexahydrate, 10 mg zinc sulfate heptahydrate, 3 mg manganese chloride tetrahydrate, 3 mg sodium molybdate dihydrate, 2 mg nickel (II) chloride hexahydrate, 1 mg copper (II) chloride dihydrate, to 1 L with distilled water.

[4] Vitamin Solution - Added to the medium by filter sterilization after other reagents were sterilized in autoclave. Weigh 100 mg thiamin hydrochloride, 100 mg pantothenic acid, 100 mg niacin, 100 mg pyridoxine hydrochloride, 10mg ammonium carbonate, 5 mg ρ-aminobenzoic acid, 0.25 mg vitamin B-12, to 1 L with distilled water.

[5] Folate-biotin Solution - 100 mg ammonium carbonate, 10 mg folic acid, 2 mg biotin, to 1 L with distilled water.

[6] Riboflavin Solution - 130 mg HEPES, 1 mg riboflavin, to 1 L with distilled water.

[7] Hemin Solution - 50 mg hemin, 40 mg sodium hydroxide, to 100 mL with distilled water.

[8] Resazurin Solution - 100 mg resazurin to 100 mL with distilled water.

Table 7.3 Organic matter disappearance (OMD%), short chain fatty acids (SCFA), branched chain fatty acids (BCFA), and total fatty acids (SCFA + BCFA) production from fermented fibers sources inoculated with dog feces for 4, 8, and 12 h.

Fiber source	MG[1]	CE[1]	BP[1]	PF[1]	SB[1]	SEM[2]	P-value
			OMD, %				
Time 4 h,	2.6^{c}	3.7^{bc}	29.8^{a}	7.6^{b}	5.8^{bc}	1.58	<0.0001
Time 8 h,	-0.8^{c}	1.8^{c}	31.9^{a}	6.5^{b}	6.4^{b}	0.98	<0.0001
Time 12 h,	1.3^{c}	2.6^{c}	38.6^{a}	9.6^{b}	10.6^{b}	0.61	<0.0001
			Short Chain Fatty Acids, mmol/g of substrate				
Acetate, 4h	0.12*^{d}	0.03*^{e}	1.48^{a}	0.35^{b}	0.22^{c}	0.028	<0.0001
Acetate, 8h	0.04*^{d}	0.05*^{d}	1.85^{a}	0.53^{b}	0.35^{c}	0.033	<0.0001
Acetate, 12h	0.08^{c}	0.02*^{c}	2.05^{a}	0.51^{b}	0.44^{b}	0.049	<0.0001
Propionate, 4h	0.008*^{c}	0.010*^{c}	0.294^{a}	0.067^{b}	0.072^{b}	0.0048	<0.0001
Propionate, 8h	0.011*^{d}	0.027^{d}	0.452^{a}	0.118^{c}	0.156^{b}	0.0081	<0.0001
Propionate, 12h	0.023^{d}	0.018^{d}	0.558^{a}	0.127^{c}	0.227^{b}	0.0123	<0.0001
Butyrate, 4h	0.004*^{d}	-0.003*^{d}	0.065^{a}	0.017^{c}	0.028^{b}	0.0025	<0.0001
Butyrate, 8h	0.004*	0.003*	0.091	0.044*	0.097	0.0306	0.1420
Butyrate, 12h	0.008^{d}	-0.003*^{e}	0.105^{a}	0.028^{c}	0.051^{b}	0.0026	<0.0001
SCFA, 4h	0.14*^{c}	0.03*^{c}	1.83^{a}	0.43^{b}	0.32^{b}	0.034	<0.0001
SCFA, 8h	0.06*^{d}	0.08*^{d}	2.40^{a}	0.75^{b}	0.55^{c}	0.051	<0.0001
SCFA, 12h	0.11^{c}	0.04^{c}	2.72^{a}	0.67^{b}	0.72^{b}	0.063	<0.0001
			Minor and Branched Chain Fatty Acids, mmol/g of substrate				
Isobutyrate, 4h	0.0007*^{c}	0.0006*^{c}	0.0058^{b}	0.0014*^{c}	0.0100^{a}	0.00069	<0.0001
Isobutyrate, 8h	0.0018*^{c}	0.0021*^{c}	0.0117^{b}	0.0030^{c}	0.0197^{a}	0.00080	<0.0001
Isobutyrate, 12h	0.004	0.019*	0.012	0.001*	0.021	0.0076	0.3281
Isovalerate, 4h	0.0039^{c}	0.0004*^{c}	0.0091^{b}	0.0068*^{b}	0.0300^{a}	0.0017	<0.0001
Isovalerate, 8h	0.004*^{c}	0.006*^{c}	0.015^{b}	0.005^{c}	0.037^{a}	0.0016	<0.0001
Isovalerate, 12h	0.0068^{b}	0.0014*^{c}	0.0089^{b}	-0.0014*^{c}	0.0357^{a}	0.0013	<0.0001
Valerate, 4h	0.00033*^{ab}	0*^{b}	0.00067*^{a}	0*^{b}	0*^{b}	0.00021	0.1705
Valerate, 8h	0*^{c}	0*^{c}	0*^{c}	0.0013^{b}	0.0026^{a}	0.00020	<0.0001
Valerate, 12h	0.0005*^{b}	0.0002^{b}	0.0002^{b}	-0.0014*^{c}	0.0031^{a}	0.00033	<0.0001
BCFA, 4h	0.005^{cd}	0*^{d}	0.016^{b}	0.008*^{c}	0.040^{a}	0.0022	<0.0001
BCFA, 8h	0.006*^{c}	0.008*^{c}	0.027^{b}	0.009^{c}	0.060^{a}	0.0020	<0.0001
BCFA, 12h	0.012^{b}	0.021*^{b}	0.021^{b}	-0.002*^{b}	0.060^{a}	0.0079	0.0030
			Total Volatile Fatty Acids, mM*g-1 of substrate				
TOTAL, 4h	0.14*^{b}	0.03*^{c}	1.85^{a}	0.44^{b}	0.36^{b}	0.036	<0.0001
TOTAL, 8h	0.06*^{c}	0.09*^{c}	2.42^{a}	0.76^{b}	0.61^{b}	0.052	<0.0001
TOTAL, 12h	0.13^{c}	0.06^{c}	2.74^{a}	0.66^{b}	0.44^{b}	0.064	<0.0001

[1] Fibrous substrate: MG: Miscanthus grass, CE: cellulose, BP: beet pulp, PF: pea fiber, SB: sorghum bran.

[2] SEM: standard error of the mean.

* P-value for the t-test greater than 0.05.

[abcde] Means in the same row with unlike superscripts differ.

Table 7.4 Short chain fatty acids (SCFA) and branched chain fatty acid (BCFA) expressed as a percentage of total fatty acids.

Fiber source	MG[1]	CE[1]	BP[1]	PF[1]	SB[1]	SEM[2]	P-value
Short Chain Fatty Acids, % of total							
Acetate, 4h	86.5^{a}	61.1^{bc}	79.7^{ab}	79.1^{ab}	61.5^{c}	4.16	0.0107
Acetate, 8h	67.4^{ab}	49.9^{c}	76.5^{a}	71.2^{ab}	57.0^{bc}	5.45	0.0357
Acetate, 12h	66.7^{ab}	45.7^{b}	75.0^{a}	77.0^{a}	56.3^{ab}	9.08	0.1561
Propionate, 4h	3.3^{b}	15.3^{ab}	15.2^{ab}	14.40^{b}	19.9^{a}	3.55	0.1930
Propionate, 8h	18.4^{b}	38.6^{a}	16.7^{b}	15.9^{b}	25.8^{ab}	4.84	0.0447
Propionate, 12h	17.9^{b}	31.2^{a}	20.4^{b}	19.1^{b}	29.3^{a}	2.71	0.0153
Butyrate, 4h	2.4	8.8	3.5	3.8	7.6	4.40	0.8031
Butyrate, 8h	4.2	2.0	3.7	11.6	7.3	3.79	0.4503
Butyrate, 12h	6.1^{a}	-4.9^{b}	3.9^{a}	4.2^{a}	6.6^{a}	1.04	<0.0001
SCFA, 4h	95.5^{abc}	91.7^{bc}	99.2^{a}	98.1^{ab}	89.0^{c}	2.13	0.0325
SCFA, 8h	89.9^{b}	90.4^{b}	98.9^{a}	98.7^{a}	90.1^{b}	0.60	<0.0001
SCFA, 12h	90.7	72.0	99.2	100.3	92.3	9.57	0.2998
Minor and Branched Chain Fatty Acids, % of total							
Isobutyrate, 4h	0.6	2.7	0.3	0.3	2.8	0.98	0.2204
Isobutyrate, 8h	2.7^{ab}	2.2^{b}	0.5^{c}	0.4^{c}	3.3^{a}	0.26	<0.0001
Isobutyrate, 12h	3.5	25.3	0.4	0.2	2.7	9.60	0.3602
Isovalerate, 4h	3.8^{bc}	5.6^{ab}	0.5^{c}	1.5^{c}	8.2^{a}	1.27	0.0097
Isovalerate, 8h	7.4^{a}	7.4^{a}	0.6^{b}	0.7^{b}	6.1^{a}	0.63	<0.0001
Isovalerate, 12h	5.4^{a}	2.3^{b}	0.3^{c}	-0.2^{c}	4.6^{a}	0.31	<0.0001
Valerate, 4h	0.15	0	0.03	0	0	0.066	0.4865
Valerate, 8h	0^{c}	0^{c}	0^{c}	0.17^{b}	0.43^{a}	0.035	<0.0001
Valerate, 12h	0.43^{a}	0.42^{a}	0.01^{b}	-0.23^{b}	0.40^{a}	0.109	0.0041
BCFA, 4h	4.50^{abc}	8.3^{ab}	0.84^{c}	1.85^{bc}	11.0^{a}	2.13	0.0325
BCFA, 8h	10.06^{a}	9.59^{a}	1.10^{b}	1.27^{b}	9.85^{a}	0.60	<0.0001
BCFA, 12h	9.3	28.0	0.8	-0.3	7.7	9.57	0.2998

[1] Fibrous substrate: MG: Miscanthus grass, CE: cellulose, BP: beet pulp, PF: pea fiber, SB: sorghum bran.

[2] SEM: standard error of the mean.

^{abc} Means in the same row with unlike superscripts differ.

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