Greg Aldrich, PhD
Research Associate Professor
Grain Science & Industry
Kansas State University
Manhattan, KS 66506

31Aug2016

Cal Boender
Renew Biomass
2520 N. Airport Plaza
Springfield, MO 65803

Dear Mr. Boender,

Please find following the cover the final report for the Evaluation of Miscanthus grass as a source of fiber in dog and cat diets. This report constitutes the agreed to work for processing animal foods for chicks, dogs, and cats and the subsequent evaluation of this ingredient for digestion and acceptability in these species. The ingredient in our evaluations was found to process in a pet food similar to beet pulp and cellulose, to be readily accepted by the animals (chicks, dogs, and cats), and generally performed in feeding assays and digestion kinetics in a manner similar to that of cellulose. There are many more fascinating application and utilization questions that remain, but for a first evaluation of this ingredient all things appear to be positive.

Thank you,

Greg Aldrich

cc.

Final Report: Evaluation of Miscanthus grass as a source of fiber in dog and cat diets

Investigator: Greg Aldrich, PhD, Research Associate Professor, Department of Grain Science & Industry Kansas State University; Phone 785-221-3979; E-mail: aldrich4@ksu.edu

31Aug2016

Executive Summary:

Chicks, dogs and cats accepted the Miscanthus grass supplemented diets as well as those fed diets containing cellulose or beet pulp and generally performed to the same level. Processing and kibble evaluation, revealed that pet foods supplemented with 10% Miscanthus grass had similar characteristics as those supplemented with beet pulp and cellulose. Dietary dry matter (DM) and organic matter (OM) digestibility of Miscanthus grass in dog and cat diets was similar to cellulose and slightly lower (as expected) to those fed beet pulp. The protein digestibility may have been influenced for dogs and cats fed beet pulp as a result of its higher concentration of soluble fiber and the ensuing fermentation in the colon and microbial protein in the feces. The Miscanthus grass and cellulose contain a preponderance of insoluble relatively non-fermentable fiber sources and appear to be quite similar as regards their impact on protein apparent total tract protein utilization. The defecation frequency was not affected by fiber source, but Miscanthus grass appeared to impact fecal scores to values closer to the "ideal" and were slightly higher to that of beet pulp and cellulose. At high levels preference in a split plate palatability tests slightly favored diets produced with cellulose and beet pulp, but at more moderate levels (5%) dogs and cats did not detect a difference. Miscanthus grass appears to be a suitable replacement for cellulose in the dog and cat diet, it was well accepted by the animals, and resulted in well-formed stools closer to ideal for the pet owner.

Introduction:

About two-thirds of U.S. households (65%) own pets, and the pet food industry in the U.S. commands a huge market worth more than $24 billion (APPA, 2016). In this mature market, sales are mostly driven by premium segments – such as health, natural, organic, fortified, functional, weight control, life stage, and gourmet. In this continually segmenting market high-fiber pet foods have an important role to play. Wood cellulose is one of the primary sources of insoluble fiber in pet foods. However, it is an expensive ingredient relative to nutritional benefit. Thus, there is a need to explore alternatives.

Fibers are used primarily in pet food for weight loss or calorie restriction diets. Wood cellulose is very popular. Pet food manufacturers use it because it is nutritionally inert (low to non-fermentable, and 'hypo-allergenic') and creates gastrointestinal bulking. It has also found favor in feline hair-ball management products as a means to move digesta through the gastrointestinal tract in a more regular and consistent manner. The level of cellulose incorporated into these diets can range from 5 to 25% depending upon the results desired and the fiber content of other ingredients in the diet.

Currently labeling restrictions do not delineate from where the cellulose is obtained. The current AAFCO (2016 official publication) definition for cellulose is “87.14 Powdered Cellulose is purified, mechanically disintegrated cellulose prepared by processing alpha cellulose obtained as a pulp from fibrous materials”. Thus, cellulose derived from alternative fibrous products could be a reasonable option and may not have to be labeled as to source or origin. This is a valuable consideration. However, for full acceptance by the market there is a need to demonstrate that an alternative has the same effect on the process, nutritional compositional, and physiological effects on animal digestion and gastrointestinal health as wood cellulose. Current cost for wood cellulose ranges between ~ $0.70 and $1.50/ lb – this reflects a substantial premium relative to food value.

As noted above, pet foods containing high levels of cellulose are typically intended for weight management, diabetes, and hairball control, to name a few. In weight control diets, the virtual indigestibility of cellulose translates into negligible food energy contribution. On a gross energy basis, cellulose is fairly similar to starch, about 4 kcal/g, but the metabolizable energy (ME; useful energy) is virtually zero. So, cellulose can be a very useful tool to help meet the low ME requirements necessary to call a pet food “light,” “lite,” or “low calorie” in accordance with the AAFCO (2016) guidelines. Without cellulose, this is very difficult to do with conventional ingredients.

The crude fiber content of commercially available powdered cellulose is around 75% of the organic matter (Kienzle et al., 2001a) and the total dietary fiber content is nearly 100%. It is mostly insoluble fiber and fermentation *in vitro* and *in vivo* is negligible for both the cat and dog (Sunvold et al., 1995a, b). When added to the diet at high levels, this amount of fiber can negatively affect the digestibility of other nutrients like protein and minerals (Muir et al., 1996; Kienzle et al., 2001b). However, this can also be exploited in the case of diabetes where cellulose has been shown to aid in the management of glucose in the diabetic dog (Nelson et al., 1991) and cat (Nelson et al., 1998; Nelson et al., 2000) when added to the diet in sufficient quantities.

Nonetheless, high levels of cellulose may be detrimental to colonocyte morphology long term (Hallman et al., 1995) by robbing these cells of critical fuel, like butyrate, due to the reduced fermentation that other more fermentable fibers could provide. In addition, while the greater indigestible mass in the stomach can act to improve conveyance of the hair mass for hairball management in cats (Davenport et al., 2002), adding cellulose to the diet increases fecal volume (Silvio et al., 2000).

Alternatively, beet pulp has become the gold standard for moderately fermentable fiber in pet diets. Much work has been conducted to evaluate its use in dog and cat foods. As beet pulp is included in the diet there is an increase in wet fecal excretion and defecations per day for both dogs (Fahey et al., 1990) and cats (Sunvold et al., 1995b). However, elevated levels of beet pulp (12.5% and 7.5% in the dog and cat, respectively) were not reported to negatively affect palatability. Like most fibers, diet digestibility declines with increasing addition; for beet pulp this occurs beyond about 5% of the diet. However, compared to non-fermentable fibers like cellulose, this decrease in digestibility is much smaller (Muir et al., 1996). Beet pulp is fermented to a limited degree in the colon but nearly a third as fermentable as extremely fermentable substrates like guar gum (Sunvold et al., 1995a). Thus, it is often described as “moderately” fermentable. This results in a slight shift in the fermentation end products to a greater proportion of the short chain fatty acid, butyrate. Butyrate is a key fuel for the colonocyte. Improvements in colonocyte microstructure health were credited to this change in fermentation end products when dogs were fed beet pulp containing diets (Hallman et al., 1995) and would tend to refute some of the anecdotal claims that beet pulp causes “plugging” of the intestinal villi.

Miscanthus (*Miscanthus giganteus*) is a C4 grass which has tremendous growth potential and produces large quantities of cellulose biomass. It grows efficiently on modest quality soils in temperate regions around the world. Commercial cultivation was first investigated to support cellulosic ethanol production during the first part of this current century for production of sustainable fuel alternatives. However, no significant breakthroughs in the conversion of cellulose to ethanol have been forthcoming; thus, sustainability for this purpose may not come to fruition. Further, fracking and other fossil fuel drilling technologies have led to lower and lower prices for crude oil and challenged prospects for ethanol. Consequently, the demand for cellulosic materials for this industry have waned. In the interim fields of Miscanthus have been cultivated and a nascent production infrastructure has been developed. Given the fossil fuels market fluctuates significantly over time, there may still be need for cellulosic ethanol and the supporting industry in the future. In the interim, expanding the infrastructure with new and expanding uses for the cellulose materials could help buffer the fluctuating demand from ethanol and aid the sustainability for the entire complex.

Currently Miscanthus grass is being grown on contract in Missouri and surrounding states, and the harvested materials have been explored as sources of carriers for feed items, pharmaceuticals, and absorbents such as kitty litter, oil dry, etc. However, these are very low value markets and may not fully capture the full potential of this crop. Miscanthus is high in fiber and by initial analysis appears to be approximately 40 to 45% cellulose (Bauer and Ibanez, 2014). While not the full 100% cellulose of a crystalline wood cellulose, it may have many of the same properties of an insoluble fiber, and could carry with it other nutritional values comparable to a fiber like beet pulp, pea fiber, or soybean hulls. All ingredients that are being added to pet diets on a routine basis to improve stool consistency, decrease caloric density, or promote digesta passage rate, and aid colonic fermentation and gastrointestinal health.

The objective of this project was to determine the impact on processing and dietary utilization of Miscanthus grass in chick, dog and cat diets.

Procedures:
Chick feeding study:

General: Miscanthus grass (Renew Biomass, Springfield, MO) was provided as coarse (passing a no. 8 screen; 2.36 mm) and fine (passing a no. 25 screen; 0.71 mm) ground material. The beet pulp was from two different sources: shreds (Midwest Agri, San Rafael, CA) which were coarse ground (to pass a no. 18 screen; 1 mm) in a hammer mill, and finely ground by a local ingredient supplier (Fairview Mills, Seneca, KS). Cellulose pellets (Fairview Mills, Seneca, KS) were ground (to pass a no. 18 screen; 1 mm). Particle size analysis was evaluated on the fiber sources using the method from the American Society of Agricultural and Biological Engineers (ASABE, 2008).

The rice, soy protein concentrate, fishmeal, ground limestone, dicalcium phosphate, salt, potassium chloride, choline, DL Methionine, L-Lysine hydrochloride (Lorstchers Animal Nutrition Inc., Bern, KS), soy oil (O.H. Kruse Feed Innovation Center, Manhattan, 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 minutes using a horizontal double ribbon 454 kg mixer, and then divided into six batches - one for each fiber source. To each batch of 114 kg the experimental fiber sources were added and mixed for 5 minutes (Table 1).

Experimental regimen:

The experimental procedures for animal use were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC). The experiment was organized in a fashion similar to Jiménez-Moreno et al. (2010). One day old male broilers (240 total) were allowed access to starter diet (23% CP corn-soybean meal starter diet) overnight with *ad libitum* access to water. The following day, chicks were weighed individually and allotted by weight to treatment into one of 30 pens (8 birds per pen) in a randomized complete block design (replicate as block; n=5). Chicks were housed under environmentally controlled conditions (temperature 24ºC, humidity 65%, and 24h light schedule) and fed experimental diets for 21 days with water available throughout the experimental period. At 7, 14, and 21 d from the start the feed intake and pen weights were recorded. Feed conversion rate (FCR) was calculated as gain per unit feed intake at each period.

Statistics:

Data were analyzed as a randomized complete block design. Pen served as experimental unit. Means were separated using a significant F using statistical analysis software on the GLIMIX procedure (SAS 9.4).

Dog and Cat Feeding Studies:

Diet Production: The fiber composition of the Miscanthus grass, beet pulp, and cellulose used to prepare the dog and cat diets were analyzed for crude fiber (AOCS Ba 6a-05), neutral detergent fiber and acid detergent fiber (ANKOM Tech.), lignin (AOAC 973.18), total dietary fiber, insoluble fiber, and soluble fiber (modified from AOAC 991.43).

The base recipe was formulated to match a “Lite” dog and cat food with protein primarily derived from animal sources. The formula was comprised of rice, poultry byproduct meal, corn gluten meal, corn, dried egg product, vitamins, and minerals to meet essential nutrient requirements (Table 2; NRC 2006). To this ration finely ground wood cellulose, beet pulp, and Miscanthus grass were added to a formulated level of 10% by mass. This ration was extruded and dried following which chicken fat and dry “digest” flavors (designed for the respective species) were added topically.

The ration was cooked on a production-scale single screw extruder (model 525, Extru-Tech Inc., Sabetha, KS) with screw profile and barrel temperatures based on preliminary studies. The target in-barrel moisture was set to 25% wet basis. The size of the kibble was controlled through the extruder die opening (7 mm and 5 mm for dog and cat, respectively). During extrusion of each batch all the parameters: screw speed (SS), preconditioner steam (PCS), preconditioner water (PCW), preconditioner temperature (PT), extruder steam (ES), extruder water (EW), knife rpm (RPM), and specific mechanical energy (SME) were collected three times (beginning, middle, and end of each production batch) using Allen-Bradley Compact Logix PLC software (Northwind Technical Services – Sabetha, KS) and considered within processing replicates for statistical analysis.

After extrusion, the product was dried in a forced air convection dryer for 30 minutes or until a target of less than 10% moisture was achieved. After extrusion and drying, each batch was coated separately with chicken fat and digest flavor in a uniform fashion using a positive displacement sprayer and in a double ribbon (140 kg) mixer. Prior to coating a sample of 12 kibbles of each batch was measured for kibble length, diameter, and weight. Kibble volume, density and sectional expansion ratio index were calculated.

Diet utilization evaluation: Dog and cat food as described above, were fed to animals in a standard digestion study. The dog study was conducted first and then 1 month later the cat study was conducted. All studies were approved by the KSU Institutional Animal Care and Use Committee. In the study, animals were fed for a period of 9 days adaption, followed by 5 days of collection. Each animal was fed each diet over the course of the experiment in a replicated Latin square design. This allows each animal to serve as its own control.

A total of 12 beagles and 12 domestic American shorthair cats were used. Each group of 4 dogs or cats were fed three diets over the course of 3 periods, and assigned randomly to replicate in the Latin Square design (Kim and Stein, 2009). Animals were individually housed with constant access to water. Lights were on a 12 h cycle with lights out from 1900 to 0700 each night. The food amounts were estimated to maintain body weight throughout the duration of the study using the NRC (2006) equations to estimate the ME of the food and food amounts. For dogs caloric intake was estimated from the equation 130*BW^0.75 and for cats as 60*BW. Animals were fed twice a day and food orts were determined 1 h after each feeding.

Following 9 days adaption sample collection began at 0800 and extended for the next 120h. Feces were collected after each meal, and whenever feces were observed throughout the day it was recorded and scored according to a 5 point scale, then collected into a whirl-pak bag and frozen for later analysis. Stools were scored using a 5 point scale: 1= watery-liquid that can be poured; 2= soft, unformed stool that assumes shape of container; 3= softer stool that retains shape; 4= hard, formed stool (ideal); 5= very hard, dry pellets and recorded in 0.5 point increments. Cat urine was collected using a 60ml syringe from collection trays under each cages into Nalgene bottles that were acidified with 1mL of 1Eq/L H_2SO_4.

At the culmination of the feeding assay fecal samples were weighed, dried, and ground to pass a 1 mm screen. Feces and food samples were analyzed for DM, OM, crude protein (CP), crude fat (by acid hydrolysis), CF, NDF, ADF, and TDF by the same procedures as described previously. Food and feces were also analyzed for the internal marker acid insoluble ash (AIA) by the method of Keulen & Young (1977).

Statistics: Data from the studies were evaluated as a replicated Latin square design; wherein, diet, animal, and period were considered in the model. The means were separated with a significant F and analyzed with the aid of statistical software using the GLM and GLIMIX procedures (SAS 9.4). Means are presented with pooled standard error of the means to provide a description of the variation, and were considered different at an alpha of 5%.

Palatability tests: Test diets were sent to Summit Ridge Farms and Kennelwood Inc. for dog evaluations and Kennelwood Inc. for a cat palatability tests. Regardless of facility, the tests included 20 animals which were offered two bowls at the same time each day and then switched in the order of presentation the next day. Each bowl contained either 400g (Summit Ridge Farms) or 650g (Kennelwood) of dog food or 120g (Kennelwood) of cat food. Animals were observed for first food eaten (first choice) and food consumed was measured as disappearance. For all the experiments, total food consumption, food consumption ratio and first choice were recorded. Differences in consumption ratio were determined by F test at Summit Ridge Farms and by t-test at Kennelwood with an alpha of 0.05 considered to be significant at either location.

Results and Discussion:

Chicks

Chicks consumed the rations quite well with no significant impediment to dietary adaptation. Feed intake was not different in period 1 (d1-7), but gain was generally greater for birds fed fiber sources relative to the sepiolite control (Table 3). Though, no difference in FCR during the period was observed. For period 2 (d7-14), chicks fed the sepiolite control diet generally had a lower feed intake than chicks fed the fiber containing diets. The FCR during this period was greatest for birds fed the beet pulp and Miscanthus grass regardless of particle size. For period 3 (d14-21), chicks fed Sepiolite had the lowest feed intake, gain, and FCR relative to the fiber containing diets. For the sum of the periods (d1-21), chicks fed sepiolite had the lowest ($P<0.05$) feed intake and gain compared to the chicks fed the fiber sources. There was no difference among the fiber sources for intake, gain, or FCR.

Those results indicate that chicks fed sepiolite had lower intake, weight gain, and FCR compared to chicks fed other diets. The fibers used all had a similar advantage regarding feed intake, weight gain, and FCR. Fiber particle size had no influence on the evaluated measurements. Chicks fed Miscanthus grass had similar performance to birds fed beet pulp and cellulose.

Food for Dogs and Cats

Diet processing results associated with the production of dog and cat diets are found in Tables 4 and 5, and kibble measurements in Figures 1 to 4. The diets were intended, within species, to be of similar composition with the exception of the fiber sources and fat and flavor were included after extrusion to not confound the impact the fibers might have on the process. Fibers are considered to be disperse phase fillers according to Guy Classification System (Guy, 2001). In other words, they are generally considered to not expand and weaken the kibble structure. That is unless they differ in some manner, which might influence the other part of the kibble such as “structure forming” functions. However, in this case all the fibers appear to have behaved in a similar manner. For all the extrusion parameters and kibble measurements there were no differences among the treatments with the exception of the beet pulp treatment for the dog diet; wherein, this diet expanded slightly more and resulted in a slightly lighter product. The differences were very small. More specifically, Miscanthus grass did not differ from cellulose in these regards. The intent of this evaluation was to confirm that the addition of Miscanthus grass to a dry mix would not unduly influence the production of a standard Lite pet food diet. The data captured from the extrusion processing in a full sized extruder indicates that there were no negative processing effects when compared to two common fiber sources in a “standard” pet food ration; even when included at a relatively high level (10%).

Other than the processing parameters, physical kibble measurements were taken and analyzed. Differently than the processing parameters for the dog foods, there were some differences in kibble dimensions and characteristics. For example, kibble length was lower and kibble diameter and sectional expansion ratio index was higher for the beet pulp diet relative to the Miscanthus grass and cellulose diets. Further, the product volume and density had a slight tendency ($P<0.10$) to be greater for beet pulp than Miscanthus grass and cellulose. This would suggest that Miscanthus grass behaves similar to cellulose in extrusion production and both were slightly less prone to expansion than the beet pulp. However, these differences were so slight that modest process modifications in production would likely compensate for any difference in a normal production setting. As a case in point, the kibbles produced for the cat foods were very similar. These data would corroborate the hypothesis that Miscanthus grass can be used as an alternative fiber ingredient by the pet food industry, with only minor to no adjustments necessary in the production and processing facilities.

The fiber composition of the three ingredient sources used to create the initial dietary formulas are presented as a composite in Table 6. Among all fiber constituents Miscanthus grass is intermediate between beet pulp and cellulose. Of particular note, the calculated cellulose content (ADF – ADL) is appreciably higher (double) in Miscanthus relative to beet pulp and roughly half that of cellulose. For total dietary fiber though Miscanthus grass is much closer to cellulose and the quantity of insoluble fiber makes up a preponderance of the fiber in a manner similar to that of cellulose. If one were to refine the small quantity of natural plant components from Miscanthus that constitute the hemicellulosic fraction, it would be virtually identical to that of cellulose. However, this hemicellulosic composition is likely to contain some elements (e.g. ferulic acid, p-courmaric acid) which might provide antioxidant benefits to the animal. It may also impact palatability slightly given the astringent mouth feel often associated with these natural plant compounds.

Apparent total tract diet digestibility was estimated in dogs and cats by two different methods: first from total collection of the feces (TFC) and second through use of acid insoluble ash (AIA) as an internal marker. Intuitively one would assume that total fecal collection to be more accurate, but anyone having conducted digestion studies with captive animals will attest that it is very difficult to actually collect all the feces excreted when they are free to move around the pen. Thus, the markers are used to provide corroborating evidence to validate the findings from total collection. The risk in this is that sometimes they yield conflicting results.

For the dogs, digestibility of DM and OM were lower ($P<0.05$) for the Miscanthus and cellulose than beet pulp (Table 7). However, CP was the direct opposite; wherein, the digestibility was higher ($P<0.05$) for the Miscanthus and cellulose than the beet pulp. This may be a function of the slightly higher soluble and fermentable fiber in beet pulp promoting the production of microbial crude protein and thereby lowering the apparent total tract digestibility. To determine if this hypothesis is correct we would need to conduct a fermentation study and (or) utilize ileal cannulated animals to isolate the effects in the small and large intestine. The crude fat digestibility was higher ($P<0.05$) for the Miscanthus grass and cellulose fed dogs in comparison to those fed the beet pulp. Crude fiber digestibility appears to be affected by dietary treatment, but this component is difficult to interpret in this sort of study and may not be as impactful to the animals nutrition as the magnitude of difference might suggest. Generally, we would assume the crude fiber digestibility of these sorts to be near zero. The ash digestibility among dogs fed the various fiber sources was not different among the treatments (average 35.9%).

The results using the AIA method to estimate fecal output in dogs was slightly different (Table 8). In this case the digestibility of DM and OM was intermediate ($P<0.05$) for Miscanthus grass between cellulose with the lowest values and beet pulp with the highest. The absolute values for each were also lower for the estimate using AIA relative to the total fecal collection. This might suggest that the AIA was providing a greater accounting for all feces excreted and confirming our concerns that TFC may not collect all feces as intended. The crude protein digestibility was lower for the beet pulp and cellulose diet and greatest ($P<0.05$) for the dogs fed the Miscanthus grass. The numeric value of all three treatments was in the same rank order as the TFC method and would continue to suggest, even though not a direct statistical link, that beet pulp is providing more soluble and fermentable fiber to the colon and that Miscanthus grass is behaving as an insoluble fiber (much like cellulose). In addition, it is worthwhile to point out that CP digestibility in the low 80% levels is common in commercial pet foods. Therefore, these values are well within what would be expected for any dietary evaluation. Using the AIA method there was no difference in crude fat digestibility among the treatments. The CF data, again are difficult to assess; especially for those values with negatives. This would suggest that the animal excreted more crude fiber than ingested. Probably more accurately, it reflects that the crude fiber is an inconsistent measure and more likely not digested to any meaningful degree in the gastrointestinal tract. The ash digestibility did differ with this method of analysis with the Miscanthus grass fed dogs, again, intermediate between the highest beet pulp, and lowest cellulose. This may not hold much nutritional meaning.

The food intake was controlled for all dogs to maintain their body weight, thus no differences between treatments were expected or intended (Table 9). Further, it must be noted that all dogs consumed the diets readily during twice a day meal feeding regime which allowed them approximately 1 h access to their food. No orts were recorded with the exception of kibbles that may have inadvertently landed in a water bowl or on the floor. One concern over the addition of fiber to a diet is an increase in stool volume and defecation frequency. However, in this work there was no change to the defecation frequency among the treatments. The fecal scores were conducted by a subjective scoring on a 5 point scale where the 3.5 to 4 point score was considered to be ideal: firm and formed. In this study the stool scores were highest ($P<0.05$) for the dogs fed the Miscanthus grass and cellulose diet versus those dogs fed the beet pulp diet (3.63 and 3.68 vs. 3.15, respectively). Thus, the dogs fed Miscanthus grass and cellulose would be considered to have produced more “ideal” feces for the consumer to handle.

Like the dogs before them, the digestibility of DM and OM for cats using TFC method was greater ($P<0.05$) for the beet pulp than Miscanthus grass and cellulose (Table 10). The crude protein digestibility data were not different among treatments. Cats are not known to have a large quantity of fermentation in the colon in a magnitude that might influence the protein digestibility like what was seen in the dog feeding assay. The fat digestibility was greater ($P<0.05$) for the cats fed the Miscanthus grass and cellulose when compared to beet pulp. Crude fiber digestibility was near zero for all treatments, and there was no difference between treatments for ash digestibility.

Using AIA as the fecal output estimate to determine apparent total tract digestibility in cats the DM digestibility was higher for Miscanthus and beet pulp than for cats fed the cellulose containing diet (Table 11). This relationship was the same using TFC, but the values among all treatments were lower. This mirrors what was observed for dogs and suggests again that the AIA method yields a higher estimate of fecal output than TFC. The digestibility of OM was higher ($P<0.05$) for beet pulp than Miscanthus grass, and this was greater ($P<0.05$) than OM digestibility for cats fed cellulose. Protein digestibility was greater for cats fed Miscanthus than cellulose and those cats fed the beet pulp containing diet were intermediate and similar to each extreme. Fat digestibility was not different among treatments (average 82.1%). Crude fiber digestibility was negative for all treatments indicating that the differences among treatments were less an issue than the methodology to assess fiber utilization. Ash digestibility was low for all treatments and does not present an opportunity to make any statements regarding the effect the dietary fiber had on their utilization.

Like the dogs, intake for the cats was restricted to maintain their body weight and did not differ between treatments (Table 12). The cats were meal fed and ate all food on offer without exception. Defecation frequency was also not different among the dietary fiber treatments. Fecal scores for the cats were lowest for the beet pulp relative to the Miscanthus fed cats and intermediate to the cats fed cellulose (2.83 vs. 3.32 vs. 3.16, for beet pulp, Miscanthus, and cellulose, respectively). Dietary fiber treatments had no impact on urine pH.

While all diets were readily consumed by dogs and cats when presented as a single choice, there is interest in determining when presented a choice whether the animals will detect a flavor or aroma difference between foods produced with a prominent contribution from a new ingredient. The common method for this evaluation is a split-plate palatability feeding assay (Aldrich & Koppel, 2015) whereby the animal is presented with two choices and is forced to make a choice. Several combinations of the diets evaluated in the digestion studies were fed in this split-plate test at two different kennels. In part to verify that the animals at one kennel were providing truly population representative results. For dogs, in the round-robin testing at Summit Ridge Farms the diets containing beet pulp and cellulose were more preferred in both total consumption and first choice than those with Miscanthus grass (Table 13). At Kennelwood the results were corroborated. While one could jump to conclusions that Miscanthus was not acceptable, one must note that there were individual dogs that preferred the Miscanthus foods. Nevertheless, as a whole the populations found something in these diets that wasn't to their customary preference. Cellulose and beet pulp are very common ingredients in pet foods, so the differences may have simply been due to the novelty of this new low-calorie ingredient.

In a follow-up study, it was shown that (Table 14) a lower level of Miscanthus grass (2.5%) when compared to cellulose did not elicit this difference and dogs found both foods to be equal in terms of consumption. However, it must be noted that at 10% of the formula, these differences were not overcome merely by increasing (5, 10, or 15%) fresh meat to the formula when all things (cellulose and Miscanthus grass levels) were the same.

Palatability assessment for cats is conducted in a similar manner, but they tend to have some different drivers for liking. In this case, unlike the dogs, cats preferred cellulose over beet pulp. However, they had the same reaction to Miscanthus grass in their kibbles at 10% (Table 15). When reduced to 2.5% and 5% the difference from their preferred fiber source (cellulose) was not so apparent and Miscanthus grass in the kibble was not an issue.

Whether these changes in palatability with a split plate method indicate an aversion to something new and whether adaptation over time could occur has not been evaluated. Clearly the dogs and cats consumed the ingredient readily in the digestion studies. But, given a choice to the new ingredient they detected a difference. Lot to lot variation is an ever present challenge in these split plate tests, so it would be valuable to continue exploring these differences as they may reveal new interpretation and potential utility for control over food intake.

Conclusions: In this series of evaluations it was observed that Miscanthus grass in chick, dog, and cat diets were readily accepted, was wholesome, and led to animal performance and diet utilization similar to that of other standard fiber sources. By analysis Miscanthus grass is relatively similar to cellulose regarding composition; especially its proportions of insoluble fiber. In digestion, Miscanthus grass is likewise similar to cellulose in the impact it has on OM, CP, and Crude Fat digestibility, impact defecation frequency, and stool scores. Finally, Miscanthus grass was readily consumed in a single-bowl feeding regime during digestion studies. However, it may suffer some slight differences in preference at higher levels (<10% formula). This seems to disappear when included at more modest levels (approximately 5%). Miscanthus grass appears to be a viable alternative to cellulose in companion animal diets.

References:

Aldrich, C.G., and K. Koppel. 2015. Pet Food Palatability Evaluation: A Review of Standard Assay Techniques and Interpretation of Results with a Primary Focus on Limitations. Animals 5:43-55.

APPA 2016. Pet Industry Market Size & Ownership Statistics. http://www.americanpetproducts.org/press_industrytrends.asp. Accessed 23Aug2016.

Bauer, S. and A. B. Ibáñez. 2014. Rapid determination of cellulose. Biotechnology and Bioengineering 111, no. 11: 2355-2357,

Davenport, G.M., G.D. Sunvold, G.A. Reinhart, and M.G. Hayek. 2002. Process and composition for controlling fecal hair excretion and trichobezoar formation. US Patent Office, Patent No. 6,383,529.

Fahey, G.C., Jr., N.R. Merchen, J.E. Corbin, A.K. Hamilton, K.A. Serbe, S.M. Lewis, and D.A. Hirakawa. 1990. Dietary fiber for dogs: I. Effects of graded levels of dietary beet pulp on nutrient intake, digestibility, metabolizable energy and digesta mean retention time. J. Anim Sci 68:4221-4228.

Guy. R. 2001. Extrusion cooking – Technologies and applications. Woodhead Publishing Limited. 217p.

Hallman, J.E., R.A. Moxley, G.A. Reinhart, E.A. Wallace, and E.T. Clemens. 1995. Cellulose, beet pulp, and pectin/gum arabic effects on canine colonic microstructure and histopathology. Vet. Clin. Nutr. 2:137-146.

Jiménez-Moreno, E., J.M. González-Alvarado, D. González-Sánches, R. Lázaro, G.G. Mateos. 2010. Effects of type and particle size of dietary fiber on growth performance and digestive traits of broilers from 1 to 21 days of age. Poultry Science 89(10):2197-2211.

Kienzle, E., B. Dobenecker, and S. Eber. 2001. Effect of cellulose on the digestibility of high starch versus high fat diets in dogs. J. Anim. Physiol. Anim. Nutr. (Berl) 85:174-185.

Kim, B. G. and H. H. Stein. 2009. A spreadsheet program for making a balanced Latin Square design. Revista Colombiana de Ciencias Pecuarias, Vol. 22, nº 4, p. 591-596.

Muir, H.E., S.M. Murray, G.C. Fahey, Jr., N.R. Merchen, and G.A. Reinhart. 1996. Nutrient digestion by ileal cannulated dogs as affected by dietary fibers with various fermentation characteristics. J. Anim. Sci. 74:1641-1648.

Nelson, R.W., C.A. Duesberg, S.L. Ford, E.C. Feldman, D.J. Davenport, C. Kiernan, and L. Neal. 1998. Effect of dietary insoluble fiber on control of glycemia in dogs with naturally acquired diabetes mellitus. J. Am. Vet. Med. Assoc. 212:380-386.

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

Nelson, R.W., S.L. Ihle, L.D. Lewis, S.K. Salisbury, T. Miller, V. Bergdall, and G.D. Bottoms. 1991. Effects of dietary fiber supplementation on glycemic control in dogs with alloxan-induced diabetes mellitus. Am. J. Vet. Res. 52:2060-2066.

Silvio J., D.L. Harmon, K.L. Gross, K.R. McLeod. 2000. Influence of fiber fermentability on nutrient digestion in the dog. Nutrition. 2000, 16:289-295.

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

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

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

Table 1. Experimental diet composition fed to chicks to evaluate growth performance and digestibility due to different fiber sources.

Ingredient	Amount (%, as-fed basis)
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
Celite	2.00
Dicalcium phosphate	1.75
Limestone	0.85
Potassium Chloride	0.50
Vitamin and mineral premix	0.25
Sodium chloride	0.23
Choline Chloride	0.22
DL-Methionine, 99%	0.21
L-Lysine-HCl, 78%	0.02

Table 2. Dietary treatments fed to cats and dogs to evaluate fibers* sources from Miscanthus, cellulose, and beet pulp in extruded diets.

Ingredients, %	Cat	Dog
Chicken By-Product Meal, Low Ash	35.22	29.05
Rice, Brewers	14.07	16.79
Corn	14.07	16.79
Wheat	14.07	16.79
Fiber*	10.00	10.00
Corn Gluten Meal, 75%	5.00	5.00
Chicken Fat	4.00	3.12
Digest, Dry, Feline	1.00	0.00
Digest, Dry, Dog	0.00	1.00
Salt	0.40	0.50
Titanium Dioxide	0.40	0.40
Potassium Chloride	0.26	0.41
Chromium Sesquioxide,	0.25	0.25
Choline Chloride, 60% dry	0.20	0.20
Vitamin Premix (Kansas)	0.20	0.15
Dicalcium Phosphate	0.20	0.00
Calcium Carbonate	0.20	0.00
Trace Mineral Premix (Kansas)	0.15	0.10
Fish Oil	0.10	0.00
Taurine	0.10	0.00
Total	**99.89**	**100.55**

Table 3. The effects of fiber source and particle size on intake, gain, and feed efficiency (FCR) of day-old chicks over 21 days.

			Beet Pulp		Miscanthus			
Item	Sepiolite	Cellulose	Coarse	Fine	Coarse	Fine	SEM	*P* =
Days 1-7 (Period 1)								
Intake (g)	108.9	130.9	114.7	114.4	127.1	111.4	7.90	0.3075
Gain (g)	100.9^{b}	108.0^{ab}	114.6^{a}	111.9^{a}	113.2^{a}	107.9^{ab}	2.86	0.0291
FCR	0.93	0.83	1.00	0.98	0.92	0.97	0.047	0.3697
Days 7-14 (Period 2)								
Intake (g)	325.8^{b}	356.9^{ab}	368.5^{a}	355.1^{ab}	375.1^{a}	345.3^{ab}	11.46	0.0730
Gain (g)	159.4^{d}	174.7^{cd}	232.2^{a}	212.8^{ab}	217.1^{ab}	197.3^{bc}	11.00	0.0008
FCR	0.49^{b}	0.49^{b}	0.63^{a}	0.60^{a}	0.58^{a}	0.57^{a}	0.027	0.0044
Days 14-21 (Period 3)								
Intake (g)	622.0^{b}	707.4^{a}	709.9^{a}	713.1^{a}	720.5^{a}	701.1^{a}	21.06	0.0294
Gain (g)	178.3^{b}	226.7^{ab}	263.4^{a}	254.9^{a}	207.78^{ab}	252.2^{a}	22.01	0.0839
FCR	0.28^{b}	0.32^{ab}	0.37^{a}	0.35^{ab}	0.28^{b}	0.36^{ab}	0.029	0.1403
Days 1-21								
Intake (g)	513.1^{c}	536.7^{bc}	600.1^{ab}	598.7^{ab}	593.4^{ab}	629.0^{a}	23.81	0.0181
Gain (g)	438.55^{c}	524.59^{b}	592.67^{a}	579.60^{ab}	538.00^{ab}	557.43^{ab}	22.17	0.0008
FCR	0.85^{b}	0.98^{a}	0.99^{a}	0.97^{a}	0.90^{ab}	0.90^{ab}	0.036	0.0731
Fiber Geometric Mean Diameter (µm)								
		107	439	227	158	101		

Means in a row with unlike superscripts differ (*P*<0.05).

Table 4. Screw speed (SS), preconditioner steam (PCS), water (PCW) and temperature (PCT), extruder steam (ES) and water (EW), knife rpm (RPM) and specific mechanical energy (SME) of dog foods produced from various fiber sources* in a high pressure and shear cooking extruder.

Dietary Treatment	SS (rpm)	PCS (lbs/h)	PCW (lbs/h)	PCT (°F)	ES (lbs/h)	EW (lbs/h)	RPM (rpm)	SME (HP/T)
Miscanthus	185	146	81.9	182	1.83	0.00	800	133
Beet Pulp	185	129	82.6	178	1.83	0.11	800	136
Cellulose	185	158	84.0	185	1.83	0.07	800	132
SEM	-	16.9	3.43	3.59	0.08	0.07	28.9	1.44
P value	-	0.50	0.91	0.38	1.00	0.60	1.00	0.22

Table 5. Screw speed (SS), preconditioner steam (PCS), water (PCW) and temperature (PCT), extruder steam (ES) and water (EW), knife rpm (RPM) and specific mechanical energy (SME) of cat foods varying fiber sources* in a high pressure and shear cooking extruder.

Dietary Treatment	SS rpm	PCS (lbs/h)	PCW (lbs/h)	PT (°F)	ES (lbs/h)	EW (lbs/h)	RPM (rpm)	SME (HP/T)
Miscanthus	185	64.8	90.8	154	38.0	0.00	1650	172
Beet Pulp	185	60.9	83.6	150	36.5	0.00	1828	185
Cellulose	185	65.1	82.8	153	49.5	0.00	1694	174
SEM	-	3.12	4.49	5.29	12.4	-	226	8.00
P value	-	0.59	0.44	0.84	0.73	-	0.85	0.53

Table 6. Fiber composition of Miscanthus grass, beet pulp, and cellulose used to produce dog and cat foods.

Item, %	Miscanthus	Beet pulp	Cellulose
Crude Fiber	45.2	18.7	72.7
Neutral detergent fiber	73.8	31.6	88.4
Acid detergent fiber	53.7	24.3	80.6
Acid detergent lignin	13.0	5.9	0.7
Hemicellulose (calculated*)	20.1	7.3	7.8
Cellulose (calculated*)	40.7	18.4	79.9
Total dietary fiber	85.5	57.7	97.5
Insoluble fiber	78.6	33.3	95.3
Soluble fiber	6.9	24.4	2.5

Hemicellulose = Neutral detergent fiber – Acid detergent fiber; Cellulose = Acid detergent fiber – Acid detergent lignin

Table 7. Apparent total tract diet digestibility based on fecal output determined by total fecal collection in dogs fed diets containing 10% Miscanthus grass, beet pulp, or cellulose.

Item, %	Miscanthus	Beet pulp	Cellulose	SEM	P
Dry Matter	78.20^{b}	81.32^{a}	77.21^{b}	1.03	<0.0001
Organic Matter	82.12^{b}	86.06^{a}	80.81^{b}	0.83	<0.0001
Crude Protein	87.88^{a}	84.48^{b}	87.58^{a}	0.92	<0.0001
Crude Fat	90.67^{a}	88.78^{b}	90.86^{a}	3.70	<0.0127
Crude Fiber	14.04^{b}	27.20^{a}	0.58^{c}	7.03	<0.0001
Ash	34.89	35.43	37.38	9.73	0.1506

^{abc} Means in a row with unlike superscripts differ ($P<0.05$).

Table 8. Apparent total tract diet digestibility based on estimates of fecal output from acid insoluble ash as an internal marker in dogs fed diets containing 10% Miscanthus grass, beet pulp, or cellulose.

Item, %	Miscanthus	Beet pulp	Cellulose	SEM	P
Dry Matter	72.20^{b}	76.55^{a}	66.66^{c}	5.43	<0.0001
Organic Matter	76.68^{b}	81.79^{a}	71.17^{c}	4.27	<0.0001
Crude Protein	84.17^{a}	79.72^{b}	81.26^{b}	5.07	<0.0020
Crude Fat	87.81	85.31	86.27	9.39	0.1508
Crude Fiber	-11.90^{b}	5.08^{a}	-48.52^{c}	16.07	<0.0001
Ash	15.09^{ab}	15.70^{a}	5.85^{b}	30.79	0.0805

[ab] Means in a row with unlike superscripts differ ($P<0.05$).

Table 9. Food intake, defecation frequency (number * day^{-1}), and fecal scores* for dogs fed dietary treatments containing 10% Miscanthus grass, beet pulp, or cellulose.

Item	Miscanthus	Beet pulp	Cellulose	SEM	P
Food Intake, g	1108.7	1116.6	1116.6	1772.9	0.9647
Defecation Frequency	2.98	2.88	3.03	3.26	0.7193
Fecal Score	3.63^{a}	3.15^{b}	3.68^{a}	0.31	<0.0001

[ab] Means in a row with unlike superscripts differ ($P<0.05$).

*Stools were scored using a 5 point scale: 1= watery-liquid that can be poured; 2= soft, unformed stool that assumes shape of container; 3= softer stool that retains shape; 4= hard, formed stool (ideal); 5= very hard, dry pellets

Table 10. Apparent total tract diet digestibility based on total fecal collection in cats fed experimental treatments containing 10% Miscanthus grass, beet pulp, and cellulose.

Item, %	Miscanthus	Beet pulp	Cellulose	SEM	P
Dry Matter	76.22^{b}	81.14^{a}	75.45^{b}	3.72	<0.0001
Organic Matter	80.47^{b}	85.85^{a}	79.37^{b}	2.8	<0.0001
Crude Protein	85.74	84.18	86.1	8.63	0.1897
Crude Fat	89.15^{a}	84.96^{b}	89.64^{a}	3.86	<0.0001
Crude Fiber	-3.79	-5.94	0.76	101.8	0.6235
Ash	30.64	31.79	32.67	63.77	0.7991

^{ab} Means in a row with unlike superscripts differ (P<0.05).

Table 11. Apparent total tract diet digestibility based on AIA to estimate fecal output of cats fed experimental treatments containing 10% Miscanthus grass, beet pulp, or cellulose.

Item, %	Miscanthus	Beet pulp	Cellulose	SEM	P
Dry Matter	69.54[a]	71.18[a]	61.98[b]	6.2	<0.0001
Organic Matter	74.46[b]	77.55[a]	67.49[c]	4.86	<0.0001
Crude Protein	81.18[a]	78.21[ab]	74.59[b]	9.66	0.0083
Crude Fat	80.03	82.81	83.6	14.37	0.1591
Crude Fiber	-34.36[a]	-68.76[b]	-56.46[b]	46.44	0.0052
Ash	9.51[a]	-8.43[b]	6.69[b]	25.44	0.0043

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

Table 12. Food intake, defecation frequency (number * day^{-1}), fecal score*, and urine pH of cats fed diets containing 10% Miscanthus grass, beet pulp, or cellulose.

Item	Miscanthus	Beet pulp	Cellulose	SEM	P
Food Intake, g	353.87	353.36	372.48	644.58	0.7883
Defecation Frequency	1.25	1.07	1.27	1.27	0.2927
Fecal Score	3.32^{a}	2.83^{b}	3.16^{ab}	1.34	0.0738
Urine pH	6.93	7.05	6.86	5.52	0.7825

^{ab} Means in a row with unlike superscripts differ (P<0.05).

*Stools were scored using a 5 point scale: 1= watery-liquid that can be poured; 2= soft, unformed stool that assumes shape of container; 3= softer stool that retains shape; 4= hard, formed stool (ideal); 5= very hard, dry pellets

Table 13. Total food consumption, food consumption ratio, and first choice of dogs at Summit Ridge Farms and Kennelwood research facilities fed diets containing 10% Miscanthus grass (M), cellulose (C) or beet pulp (B) in a 2 day 20 dog split plate palatability test.

Test ID	Total Food Consumption		Total Consumption Ratio		First Choice	
			Summit Ridge Farms			
B vs C	5070	4074	1.24	1	21	19
B vs M	7308	2150	3.40*	1	27	13
C vs M	6632	2251	2.95*	1	28	12
			Kennelwood			
B vs C	6323	9288	0.68	1	16	24
B vs M	11654	3829	3.04*	1	29	11
C vs M	10825	3226	3.36*	1	32	8

*P<0.05 for total consumption ratio only

Table 14. Total food consumption, food consumption ratio, and first choice of dogs at Summit Ridge Farms research facilities fed diets containing 2.5% Miscanthus (M) or Cellulose (C), and (or) Dogs fed fresh meat (FM) diets (meat included at 5, 10, and 15% of the formula) and 10% Miscanthus grass (M), or cellulose (C) in a 2 day 20 dog split plate palatability test.

Test ID	Total Food Consumption		Total Consumption Ratio		First choice	
2.5% Fiber C vs M	3017	4190	0.72	1	16	24
5% FM, 10% Fiber C vs M	5754	2208	2.61*	1	24	16
10% FM, 10% Fiber C vs M	6901	1844	3.74*	1	34	6
15% FM, 10% Fiber C vs M	6039	1808	3.34*	1	31	9

*P<0.05 for total consumption ratio only

Table 15 Total food consumption, food consumption ratio, and first choice of cats (20) fed diets (2 day switch back split plate palatability test) containing 10% Miscanthus grass (M), cellulose (C), or beet pulp (B) at Kennelwood research facilities, or cats fed diets containing 2.5% or 5% cellulose, and Miscanthus.

Test ID	Total Food Consumption		Total Consumption Ratio		First Choice	
			Kennelwood			
C vs M	1541	219	7.04*	1	33	7
B vs M	1293	350	3.69*	1	27	13
B vs C	344	1430	0.24*	1	11	29
			Summit Ridge Farms			
2.5% Fiber C vs M	874	1085	0.81	1	15	25
5% Fiber C vs M	716	552	1.30	1	20	20

*P<0.05 for total consumption ratio only

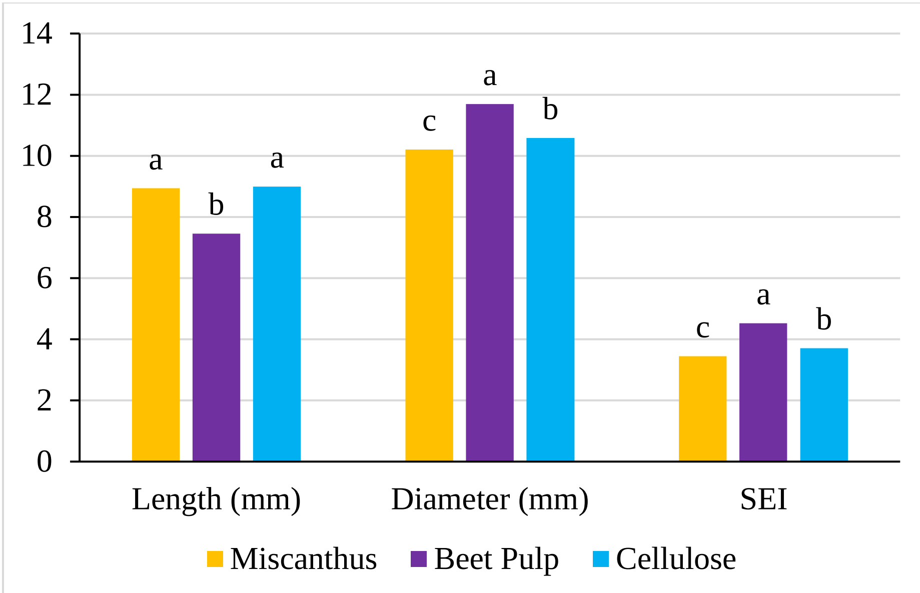

Figure 1. Kibble length (mm), diameter (mm), and sectional expansion ratio index (SEI; mm^2/mm^2) of extruded dog food produced with 10% Miscanthus grass, beet pulp, or cellulose (^{abc} bars with unlike superscripts differ; P<0.05)

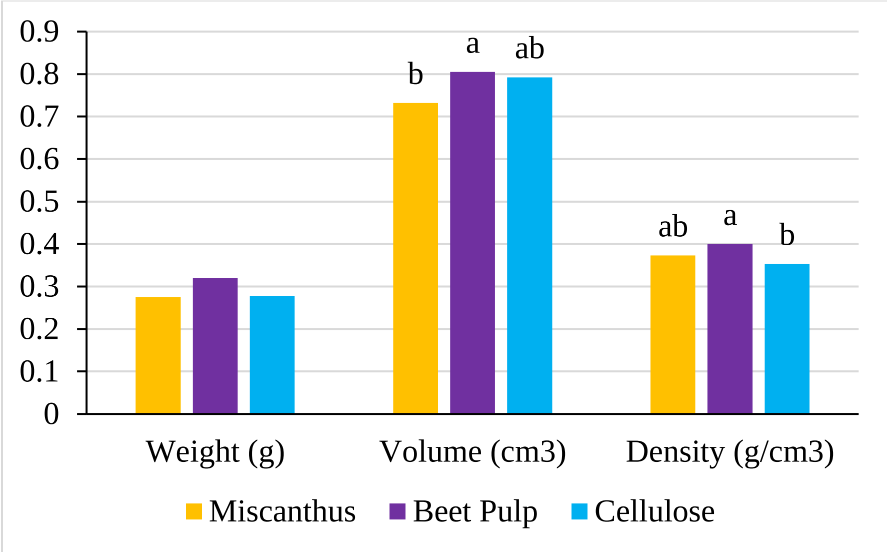

Figure 2. Kibble weight (g), volume (cm^3), and density (g/cm^3) of dog food produced with 10% Miscanthus grass, beet pulp, or cellulose ([ab] bars with unlike superscripts differ; P<0.05).

.

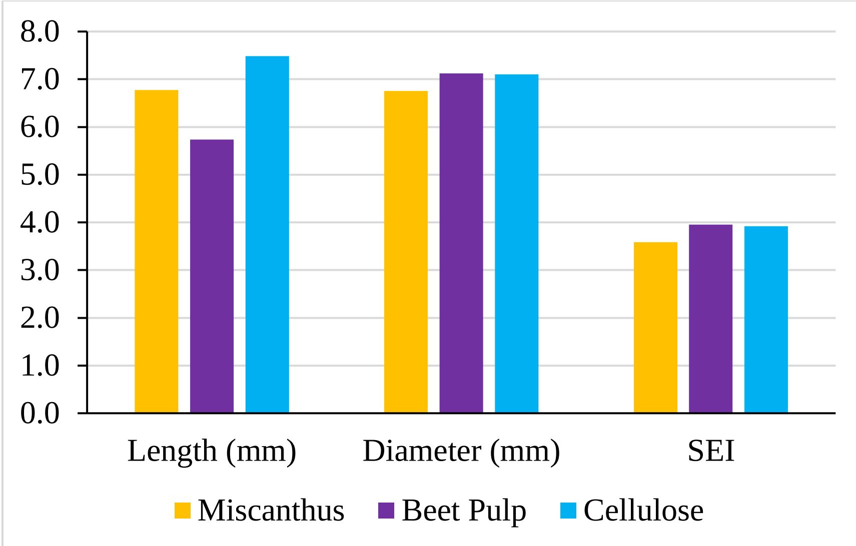

Figure 3. Kibble length (mm), diameter (mm), and sectional expansion ratio index (SEI; mm^2/mm^2) of cat food produced with 10% Miscanthus grass, beet pulp, or cellulose.

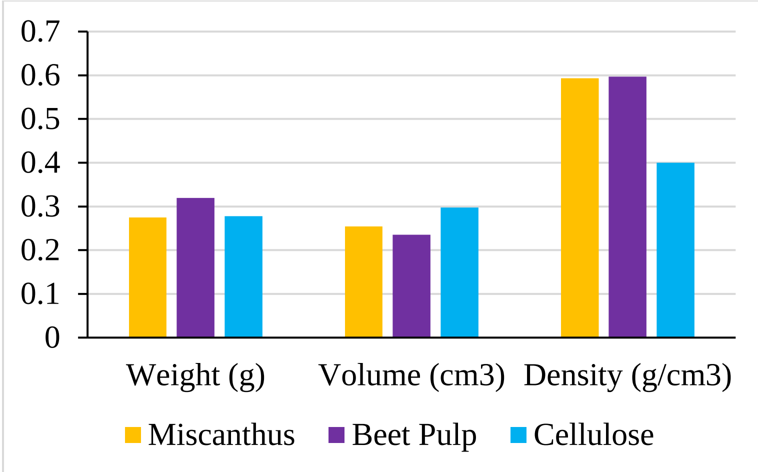

Figure 4. Kibble weight (g), volume (cm^3), and density (g/cm^3) of cat food produced with 10% Miscanthus grass, beet pulp, or cellulose.