## **Introducing New Profit Streams for Grain Sorghum**

Milling Characteristics and Inclusion Considerations for Use in Pet Foods



#### **Conducted by:**

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### Summary

Whole sorghum was milled into sorghum flour and sorghum millfeed (bran, shorts, red-dog, and germ) at the Hal Ross Flour Mill and evaluated for yield and nutrient composition. These fractions were then subsequently incorporated at <65 percent into an extruded dog food diet at the BIVAP center at Kansas State University. Product expansion was slightly lower in the diet containing the millfeed, but whole sorghum and sorghum flour produced kibbles requiring similar process settings and outcomes to a control diet containing a combination of wheat, rice, and corn. These diets were then fed to dogs in a digestibility study and it was determined like processing that the whole sorghum and sorghum flour containing diet had digestibility and stool consistency similar to that of the control, but; dogs fed the diet containing the millfeed had lower overall digestibility, larger, harder, and more frequent stools. These dogs also had a two-fold higher concentration of circulating antioxidants (ORAC value). This was considered a substantial finding. As it relates to sensory attributes of the sorghum treatments there were no major differences detected by the trained panelists for the sorghum containing diets for aroma, flavor, or texture, but they noted the different visual effects from the smaller (less expanded) millfeed containing diet. In conclusion, the milling of whole sorghum into fractions resulted in two unique products that should find favor as novel ingredients to pet food and processed in a predictable fashion through flour milling, and pet food extrusion, were readily eaten by the dogs, were comparable to a control and whole sorghum in trained sensory panelists, and overall digestibility was comparable for the flour. Improvements will be needed on the amount of the millfeed incorporated into the diet such that digestion is not compromised, but the benefit from the elevated circulating antioxidants are retained.

#### Introduction

The overall goal of this project was to explore the opportunity to create new sorghum based ingredient streams that could be offered to the pet food industry as new items and ultimately increase the use of sorghum. Of course sorghum is an important crop in the U.S., but mostly as a feed stock for livestock and (or) ethanol production. But, little finds its way into the growing pet food market. There may be opportunities with this sort of an approach to change that.

The pet food market is a vibrant component of the agriculture and feed landscape with an estimated \$23 billion in annualized sales in the U.S. alone. This would translate into approximately 8.5 MMT, approximately 40 percent of which would come from grains produced in the U.S. Sorghum should have a larger proportion of this volume than it does.

The pet food industry is a marketing driven consumer packaged goods category and in a constant search for new and alternative ingredients for which to promote their attributes, expand their market, and differentiate their products. Discovery of "new" ingredients is vital, and "defensible claims" are highly valued and rewarded in this high-margin market.

Whole sorghum is not new to this market, but sorghum components could be a significant new and exciting ingredient(s) entrant if there was a sufficient amount of supporting information and instruction on their use.

#### **Background and Relevance**

**Sorghum:** Globally, sorghum is the fifth most important grain (around 54 MMT produced annually) with the U.S., India and Nigeria being the top three producing countries (FAOSTAT3, 2011). Production in the U.S. is centralized around Texas, Oklahoma and Kansas (the leading state in its production). Sorghum is non-GMO, "green," low water use crop that uses less herbicides and pesticides than corn, and fits well with crop rotation systems on the farm. In the pet market, these are all positive attributes for consumer decisions. Despite this global reach and supply-side sustainability, the value of sorghum is little recognized by pet food marketing representatives. The issue is not a matter of nutrition.

**Nutrient composition and utilization:** Nutritionally, sorghum should be a good fit for pet food. The nutrient profile compares favorably to corn, with starch being the largest portion (around 75

percent). Most of the starch is in the form of amylopectin (70-80 percent). The protein content is slightly higher than corn at or above nine percent with lysine and threonine being the first and second limiting amino acids. The fat content of sorghum is slightly lower (about three percent) than corn, which is in part responsible for its lower metabolizable energy. The essential fatty acid (for dogs and cats) linoleic acid (C18:2n6) comprises greater than half the total fatty acids and another third comes from oleic acid (C18:1n9; not an essential fatty acid for pets). Less than three percent of the total fatty acids come from the omega-3 linolenic acid (C18:3n3). The fiber fraction (crude fiber 2-3 percent) is primarily insoluble with measurable amounts of lignin (primarily ferulic and p-coumaric acid) in the seed coat. The mineral (ash 1-2 percent) and vitamin content are comparable to many other cereal grains.

**Sorghum for dogs and cats:** Several researchers have evaluated the use of whole sorghum in extruded dog and cat diets. In dogs, the extent of starch digestibility was reported to be similar to that of other grains like corn or rice (Carciofi et al., 2004; Twomey et al., 2002; Murray et al., 1999). The in vitro work of Murray et al. (2001) suggested that the rate of starch digestion might be slower due to the composition of the sorghum starch. Corroborating evidence of this, albeit indirect, has been reported with intravenous glucose tolerance tests. In this case, studies with dogs (Carciofi et al., 2004; Sunvold and Bouchard, 1998) and cats (Bouchard and Sunvold, 2000) fed extruded sorghum-containing diets, had lower concentrations of glucose, time to peak glucose was longer and the insulin response curve was dampened after a test meal containing sorghum when compared to diets containing rice or corn. In essence, sorghum demonstrated a lower glycemic index (GI). A low GI claim has become a very popular attribute for pet food marketers.

Beneficial condensed tannins: Sorghum processes much like corn or rice and expands well in extruded diets. For specialty markets, sorghum is gluten free and is currently non-GMO. However, despite what appears to be a generally good report card, there are very few pet food companies that use or promote sorghum as part of their foods. The reason for this lies with sorghum's limited name recognition by consumers and a reputation in some feed sectors for being slightly lower value relative to corn. This reputation has been earned from some less than stellar feeding studies from the past in which digestibility and performance of livestock compared unfavorably to corn. This contradiction for livestock feed results with the results noted above for dog and cat food rests at the seed surface. As is commonly recognized, for the commercial trade, sorghum is classified into several groups based primarily on the seed coat (pericarp) color. In its simplest interpretation, sorghums are either "tannin" (brown), white or a couple of incremental combinations of the two (Rooney and Serna-Saldivar, 2000). The tannins, though not actually tannic acid like the name would suggest, are more accurately proanthocyanidins or for simplicity "condensed tannins". All sorghums contain these condensed tannins – some more than others. These condensed tannins are a cauldron of different polyphenolic compounds produced as secondary metabolites by the plant. These compounds support the plant's natural defense system against insect predation and mold infestation. Some sorghum varieties have been selected to better exploit these benefits (i.e. brown "bird resistant milo"). However, there is an offsetting drawback as some of the condensed tannin sub-fractions precipitate proteins, inhibit digestive enzymes (amylase inhibitor, trypsin inhibitor) and bind (chelate) trace minerals. Because of this it has been common to consider condensed tannins as anti-nutritional factors to avoid, especially, the "tannin" (brown) sorghums. In addition, tannins have been related to astringent mouthfeel and bitter taste in foods manufactured from sorghum (Brannan et al., 2001). It has been found that astringency and bitterness in sorghum foods develop at different speed and that the astringent sensation lasts longer than does bitterness (Kobue-Lekalake et al., 2012). This aspect may influence animal acceptability of feeds manufactured with sorghum flour and should be studied via human sensory analysis and palatability testing by pets.

The upside is that not all condensed tannins are the same. Recent research has shown that sorghum proanthocynadins may impart health benefits. For example, the hydroxycinnamic acid and flavonoid fractions of these condensed tannins have anti-tumor (esophageal, colon), anti-viral (HIV), melanogenic and satiety effects. The flavonoid pigments (blues, purples and reds) are process stable natural food colorings. Furthermore, the antioxidant properties of many of the phenolic compounds impart to sorghum a greater antioxidant capacity than found in some fruits and vegetables commonly

thought to be antioxidant rich (e.g. plums, broccoli, carrots, etc.; Dykes and Rooney, 2005). Since condensed tannins are not a single compound, but rather a host of many, there is an opportunity to identify and select sorghums for beneficial properties at the expense of the anti-nutritional properties and/or to create sub-fractions that concentrate certain elements and provide added value. It is this latter approach that we propose are in the process of evaluating.

**Sorghum potential:** Thermal processing, such as extrusion and steam flaking, and chemical treatment with alkali can improve performance of high condensed tannin sorghums. Currently, low condensed tannin varieties (white, red and yellow sorghums) are more common and can be used effectively in dog and cat diets as a key ingredient in mainstream conventional diets as well as a vital part of a diabetic and weight control regime. On the horizon, there may be opportunities to exploit the antioxidant and nutraceutical benefit of sorghum in pet food once the condensed tannins story is better clarified. If this comes to pass, sorghum might emerge as the next "hot" new novel carb. Our recent research has shown that consumers are willing to accept foods that include sorghum better if these foods include some other characteristics, as well, such as grown in the U.S., high in antioxidant content, or most importantly, sensory characteristics (Vazquez-Araujo et al., 2012).

It is important to note that most of the key attributes of sorghum identified above are for component parts of the seed; whereas, all the research reported thus far for pets has been done with the whole seed. In other words, the missing component to making a value added sorghum utilization improvement is to process the seed into components, which will improve its prospects for inclusion into pet foods and other specialty feeds. This may grab the attention of the market and stimulate new use of sorghum as a higher value "health" product.

## **Goals and Objectives**

The overall goal of this project is to understand sorghum component characteristics during milling and processing into pet food and their utility from an animal feeding perspective.

The following are the objectives of this study:

1. To evaluate dry milling processes on yield characteristics of sorghum fractions (flour, bran, germ) as value-added ingredients in pet foods,

2. To determine the impact of various component parts (flour, bran, germ) from sorghum on processing characteristics in standard pet foods, and

3. To determine the effects of varying levels of sorghum flour, bran, germ content in a standardized pet food on indices of animal utilization, palatability, and pet owner acceptance.

#### **Methods and Materials**

Sorghums selected for this study were obtained from locally grown supplies during the 2013 and 2014 crop year. Sorghum seed has three major components: the endosperm, germ and pericarp. All sorghum seeds contain a testa, which separates the pericarp from the endosperm. If the testa is pigmented, sorghum will be red or brown and will contain condensed-tannins, which are rich in phenolic acids. These confer antioxidant protection properties to the grain (and potentially to the consumer). The endosperm is composed of the aleurone layer, peripheral, corneous and floury area. The corneous and floury endosperm cells are composed of starch granules, protein matrix, protein bodies and cell walls rich in  $\beta$ -glucans and hemicellulose. Commercial milling to separate sorghum into various fractions has been done previously, but typically to extract the flour portion alone. These latter components may be more valuable if processed and characterized properly and consistently.

**Preliminary milling evaluations:** Initial pilot-scale milling was undertaken in July 2014 using locally produced red sorghum grown during the 2013 crop year. This step was undertaken to evaluate the capabilities of the Hal Ross Flour Mill (HRFM) as a first production test milling sorghum as a grain since the plants commissioning. Yield and composition of sorghum and fractions were analyzed for proximate composition (Table 1). Fractions from this test were not used for experimental diet production. However, the flour was utilized for another pet treat product

development (See proceedings Pet Food Workshop 2015; Innovation Pet Treats, Olathe, KS).

For laboratory-scale milling, four samples of 1,000 grams of red sorghum were milled at lab-scale bench top roller mills (model Ross E-1, Ross Machine & Mill Supply Inc., Oklahoma City, OK) at Shellenberger Hall, Kansas State University, Manhattan Kansas (Table 2). In this procedure, the red sorghum was cleaned and conditioned to 16 percent moisture content prior to lab milling. The lab milling was set-up and designed to mimic the HRFM process. The sorghum first passed through the break passages: break 1, 2, 3, 4 and 5. The break passages gradually removed the endosperm from the bran, and after the fifth break there was little to no endosperm to remove, so the sorghum endosperm was transported to the reduction system and the bran exited the milling process. The endosperm on the reduction system was ground into flour particle size through multiple grinding and sifting steps. In total, four replicates were completed.

**Sorghum milling for diet production:** Sorghum was purchased from 2014 crop production and milled on April 28, 2015, at HRFM. A total of 100 bushels (ca. 5,600 lbs / 2,545 kgs) of red sorghum were purchased from AgMark LLC on March 31, 2015. An amount of 3,354lbs (1,525 kgs), approximately 2/3 of the whole sorghum purchased, was milled in order to separate flour, bran (mill-feed), and germ. The remainder (1,200 lbs / 545.5 kgs) was ground in a hammer mill using a #16 standard sieve (1.191 mm) to produce whole sorghum meal for diet production of the WSD treatment. The whole sorghum flour was passed through a sifter after being ground on the hammer mill. The sifter was sized with a 560 micron screen. Material passing through the sifter was collected as ground whole sorghum while the scalps of the sifter went back to the hammer mill for further grinding.

In a preliminary step to milling, the sorghum was cleaned of impurities, such as weed seeds, straw, soil particles, spoiled decayed grains, dust and other incidentals. This cleaning step is based on the cereal's kernel size, shape and flow in air, friability and specific gravity.

The sorghum was received at 14 percent moisture and conditioned with water to raise the moisture level to 16 percent. Increasing moisture content to 15–17 percent facilitates the separation of starchy endosperm cells from germ and hull. This conditioning the grain toughens the bran layers and softens the endosperm to aid in a more clean separation of bran and endosperm during milling.

Whole sorghum was passed through the roller mills in order to reduce particle size and open the kernel by pressure and shear forces. Sorghum flour, germ and bran were separated according to particle size using sieves in a continuous flow gyratory sifter. Rollers were matched to the product needed - their size, surface flutes, rotation velocity, gap between pairs of rollers rotating in opposite directions at dissimilar speeds. Sorghum milling is a process that involves many steps that are part of the grinding, sifting and purification. The grinding passages consists of breaks, sizing, collection and middlings.

The process is summarized as follows: whole sorghum was passed through first and second break at approximately 20 percent release on first and 65 percent release on second break. The ground stock was then sifted to separate by particle size. Coarser particles consisted of mainly bran with endosperm still attached. Finer particles were a mixture of smaller endosperm chunks and small broken bran particles, which went to purifiers to be cleaned. The coarser particles went to the third break, which had a target release of 60-80 percent. The bran particles then continued through the break passages where the corrugated rolls gradually scraped away and removed endosperm from the bran. There were five total break passages where the removal of endosperm from the bran occurred. After fifth break, the bran was clean of all endosperm, at which point it is removed from the milling system through the sifter and sent into a collection bin. The hull and a substantial part of the aleurone layer were removed in the form of bran. A larger portion of the sorghum germ was lost with the bran after the break passages.

The purification step consisted of cleaning small bran particles from the endosperm particles and sending dirty compound particles of endosperm and bran to the sizing system. This was accomplished with purifiers through sifting with the aid of air acting on the stock. The air lifts the light particles (bran), which "tail" over the end of the screens while the more dense particles (endosperm) fall through the screen and out the bottom of the machine. From the purifiers, the dirty compound particles are sent to the sizing system where the particles are very gently ground to break apart the compound particle of endosperm and bran. The endosperm continued on to the reduction system while the branny particles went to the collection passages.

From the purifiers and sizing system, the clean endosperm particles went to the reduction system where the particle size was reduced by grinding into flour. There were six reduction grinding passages in the Hal Ross Mill where the endosperm was ground into flour. After each grinding passage, the material was sifted to separate by particle size. The fine material sifted off went to flour, and the coarse material was sent to the next reduction passage in order to further reduce the particle size into flour. A portion (ca. 5–8 percent) of the starch granules was mechanically damaged during milling at the reduction passages.

Quality and tailing are an intermediate step of flour milling process, representing the collection system. These passages are bran rich streams from the milling process that still have some usable endosperm and contain the remaining germ. The streams are too dirty to be send into the reduction passages, so the product is ground on the collection passages to reclaim any remaining flour. The bran is sifted off and sent to the bran bin. Sorghum germ was removed on sifters as well after the collection passages, quality and tailing, flatten the germ down into a large flake. A milling diagram and mill settings used to produce sorghum fractions form this projects were added to this document to help understand the process (Figures 1 and 2).

**Diet development:** Three diets were formulated to be iso-nutritional based on carbohydrate, lipid, protein, and mineral and vitamin content using sorghum fractions produced during the milling tests above: whole sorghum diet (WSD), sorghum flour diet (FLD), sorghum bran enriched mill-feed diet (MFD), and a control diet made with corn, wheat and rice (CON; Table 4). A portion of all diets produced were dosed with chromic oxide and titanium dioxide as external markers in order to calculate nutrient digestibility in dogs. The other portion of all diets that did not contain markers were used for the sensory evaluations at the KSU Sensory Analysis Center (Ice Hall, Kansas State University, Manhattan, KS) and palatability testing in-home and at a commercial kennel.

Sorghum was acquired as noted above. Rendered chicken fat was purchased (IDF, Springfield MO) and was preserved with BHA, propyl gallate and citric acid. All other ingredients were purchased from a local mill that supplies ingredients to the pet food industry (Fairview Mills L.P., Seneca, KS).

**Mixing and Grinding:** The mixing, grinding and extrusion took place in the Bioprocessing and Industrial Value Added Program (BIVAP) facilities at Kansas State University, Manhattan, KS. Ingredients were weighed on a digital scale and added to a 500lb paddle mixer in the order of the ingredient with the highest to the least percentage. All micro ingredients, including the markers, were weighed together and added last to the ration that was mixed for 5 minutes. All the ration mixed was overestimated in order to avoid time waste in case any complication was to occur. Also, the extrusion process needs some material that will be wasted for warm up (approximately 30 minutes) and for the transition period in between diets extruded. In total one batch of 500lb for each of the diets with markers (CON2, WSD2, MFDLD2, FLD2) and the control diet without marker (CON1) were mixed and one batch of 300lb for diets MFDLD1, WSD1 and FLD1 (no markers) were mixed. Simultaneously with the mixing, rations were ground in a hammer mill (Fitzmill, Elmhurst, IL) with a 840um screen size. The particles were finely ground in order to facilitate extrusion and digestibility.

**Extrusion Processing:** The four treatments were extruded in duplicates by 1) presence of chromic oxide and titanium dioxide markers for the digestibility study, and 2) without markers for sensory attributes. The extrusion was done at BIVAP extrusion lab (May 13 and 14, 2015). The treatments were produced on a single screw extruder (Model X-20; Wenger Mfg, Sabetha, KS) using a typical pet food screw profile (Figure 3).

The extruder screw profile includes: Inlet screw, single flight full-pitch screw, small shear lock, single flight full-pitch screw, small shear lock, single flight screw, medium shear lock, double flight single pitch screw, large shear Lock, double Flight Cut Cone Screw.

Bulk density was measured using a 1L cup and a scale with 0.01g sensitivity. An empty 1L cup was used to tare the scale. After exiting the end of extruder, the cup was filled in with the pet food and

the top layer of the product was gently leveled of by hand. The full cup was weighed and bulk density was recorded.

Post-extrusion, kibbles were pneumatically conveyed to a dual pass dryer/single pass cooler (Model 4800; Wenger Mfg, Sabetha, KS). The dryer was set at 123.8°C and kibbles were conveyed eight minutes per pass (16 minutes total) and five minutes through the cooler. The final moisture goal was for the food to be below 10 percent. After drying, all the food was conveyed to a coating tunnel where the chicken fat was applied according to each formulation. All kibbles fell into a tub and they were manually placed in 20lb (9.1 kg) poly-lined Kraft-paper bags.

Samples of kibbles before and after coating were collected for further analysis. Five kibbles from each time period of each diet before coating were randomly selected. Using a pair of digital calipers kibble diameter and length were measured, followed by weighing on a digital scale with 0.0001g sensitivity (Explorer EX324N, Ohaus Corporation, Parsippany, NJ, U.S.A.). The diameter, length and mass measurements were used to determine piece volume and density using the calculations below:

Piece volume=  $\pi^*$ (piece diameter)\*(piece length)/4,000 (cm3)

Piece density= (piece mass)/(piece volume) (g/cm3)

Texture analysis was performed using a TA-XT2 Texture Analyzer (Texture Technology Corp., Scarsdale, NJ, U.S.A.), equipped with 50 kg load cells. A 25 mm cylindrical probe was used to apply uniaxial compression on five kibbles per time period of each replicate within diet, at a pre-test speed of 2 mm/s, test speed of 1 mm/s, post-test speed of 10mm/s, and strain level of 50 percent. The protocol used was a modified version of what was described by Dogan and Kokini (2007). Kibble hardness was considered to be the peak force (N), and energy needed to compress the kibbles to 50 percent was the calculated area under the curve (Nxmm) of each compression signature.

**Digestibility determination:** Twelve intact Beagle dogs, eight males and four females, all young adults (1-3 years old) were housed individually in twelve cages (1.83m x 1.20m) equipped with an acrylic-mesh floor and three-piece pan underneath to allow separation of feces and urine. The study was conducted over fifty-six days at the Large Animal Research Center (LARC) at Kansas State University, Manhattan, Kansas. There were four periods divided into adaptation period (nine days) and collection period (five days). All animal testing was approved by the Kansas State University Institutional Animal Care and Use Committee prior to the conduct of the study. This was the first digestibility study using dogs ever conducted at Kansas State University.

Dogs were fed one of four nutritionally complete and balanced dietary treatments (Tables 4 and 5) during the study: a rice, wheat and corn based (CON), whole sorghum based (WSD), sorghum flour based (FLD), and a sorghum mill-feed enriched with bran based diet (MFD). All diets included 0.4 percent of titanium dioxide and 0.25 percent of chromic as markers. The digestibility study was conducted as a replicated Latin square design. Each group of three animals was randomly assigned to a specific diet during each period, so that all dogs ate all diets at the conclusion of the study.

As a starting point to determine dietary intake the dogs' daily metabolizable energy (ME), requirements were calculated using the activity coefficients and empirical equations for laboratory kennel dogs or active pet dogs: 132\*BW^0.75 (National Research Council, 2006). The finished diets ME's were calculated using the predicted equations for ME in dog food (National Research Council, 2006). Dividing the daily ME required by the predicted food ME a daily food intake (in grams) was estimated. Based on dog weight, the food offered was adjusted throughout the study.

Dogs had free access to water at all times and were housed in a climate-controlled building with a temperature of 22-23°C. Dogs were identified by ear tattoo. The four test diets were placed in rubber storage bins inside the building where the dogs were housed. Each food tub had a colored tag corresponding to the diet each dog ate during each period. A worksheet with food weights per dog was created and diets were weighed according to computed amounts, then given to dogs at feeding times. After 60 minutes the orts were removed, weighed, amounts recorded, and then discarded. Weights before and after feeding were written in the laboratory notebook.

During the collection period (day 9 -14), dogs were fed at 0800. Any remaining orts, including kibbles on the floor were collected after one hour (0900). Wet orts were placed in separate bags to be dried and weighed later. Orts were weighed on a digital scale (model N1B110 Navigator, Ohaus Corporation, Switzerland) and recorded.

There were four collection periods in total during which feces and urine were collected at 0800 on the first day and concluding at 0800 on the last day, for a total of five days (120h). On the first day of collection, feces and urine were removed (not collected yet) at 0730 and the pens were cleaned. Everything dogs excreted after 0800 was collected. On the second, third and fourth days, dog feces and urine were collected at 0730, food and water was provided at 0800 and orts, if any, were harvested at 0900. At 1100, pens were properly washed and in the afternoons feces and urine were collected at 1700 and orts harvested at 1800. Every time there were feces in the cages, it was collected, placed in the appropriate sample bag, scored for consistency, and placed in the refrigerator temporarily until put into a freezer (-15°C). The number of defecations were recorded and stools were scored using a 5-point scale, wherein: 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. The scale had 0.5 points increments.

After collecting feces, urine was extracted from each catch-pan below the wire pen flooring using a 60ml syringe (one syringe per dog). Total urine collected during each period was poured into 1L sterile labeled jars with 1mL of sulfuric acid solution (1Eq/L) stored in the refrigerator then composited across period and subsampled (50mL) in duplicates into acrylic tubes and frozen (-15°C). Unfortunately urine could not be collected from all dogs due to caging limitations, so no real measure could be taken from that.

On the fifth day of the collection period, dogs were weighed, assessed for body conditions score (BCS) and manually restrained for blood collection. Blood (6mL with a 22g needle) was drawn by brachial artery via venipuncture. The ~ 6mL blood was immediately divided in half with a portion paced into tubes with and without EDTA then placed in on ice. At the culmination of blood collection tubes were centrifuged at 2000G for 10 minutes to separate plasma (EDTA tubes) and serum (non-EDTA).

Plasma was analyzed for antioxidant activity using the oxygen radical absorbance capacity (ORAC) protocol. An ORAC kit was purchased from Cell Biolabs, Inc. (San Diego, CA). The procedure consists of measuring the fluorescein depletion by a free-radical over time (kinetic fluorescence assay) using a plate reader (Gen5TM, Biotek® Instruments, Inc.Winooski, VT). The amount of antioxidants in the sample are directly proportional to the intensity of fluorescence.

Sensory Analysis: For the sensory analysis all samples were served at room temperature. Dry dog food samples were served in 100 mL (~3.25 oz) cups for appearance, flavor, aftertaste and texture evaluation. Medium covered snifters were used to evaluate aroma of the samples. Three grams of each sample were placed in the snifter and then covered with a glass lid. Five highly trained panelists from the Sensory Analysis Center at Kansas State University (Manhattan, KS, USA) participated in the study. For this project, the panelists received further orientation to dry dog food using samples included in the study. Each product was evaluated for aroma and flavor using attributes from a specific dry dog food lexicon developed by Kansas State University (Di Donfrancesco et al., 2012). The panelists had two orientation sessions of two hours each to develop a ballot and become familiar with the sensory characteristics of samples. Five panelists evaluated four samples and scored intensity for appearance, aroma, flavor, aftertaste and texture attributes independently in triplicate in a randomized order. A total of four samples were evaluated during each 1.5 hour session. The intensity of scores were based on a 0-15 point scale with 0.5 increments (0.0 =none; 0.5 - 5.0 = slight; 5.5 - 10.0 = moderate; 10.5 - 15.0 = high). Data were analyzed by analysis of variance to determine significant (p<0.05) differences among the sample set. Statistical software (SAS 9.3; Cary, NC) was used to perform all statistical analysis.

Acceptance of dry dog food made with different fractions of sorghum. Consumers Screening: 105 consumers were selected from a total of more 500 consumers screened. All the participants were dog owners, fed their dog dry food, and directly involved in the purchase and selection of the

food. Samples serving was administered as a single cup of each sample diet was served at room temperature into a 8oz Styrofoam bowl covered with a lid. The lid was removed by participants during the evaluation. Each consumer evaluated four dry dog food samples served in a randomized order. Evaluation was conducted in one session. A total of 15 session were held. Data were collected using iPads tablets and Red Jade software. Consumers were asked to indicate a overall liking score, overall appearance liking score, color liking score and an aroma liking score for the samples, using a 1-9 hedonic scale (1 – dislike extremely, 9 – like extremely). Analysis of Variance to determine significant (p<0.05) differences among the sample set. SAS software (9.3 version) was used for the statistical analysis.

In addition to the flavor profiles using descriptive sensory analysis (Di Donfrancesco et al., 2012), the chemical constituents of the main aromatic compounds (Koppel et al., 2013) will be determined. The dietary treatments will be subjected to a consumer acceptance study to determine if aroma or appearance of dog foods manufactured with sorghum in relation to dog foods manufactured with a control grain such as rice have an effect on consumer opinion. Finally, experimental dog food will be subjected to a standard two-bowl dog palatability test. Information collected from the descriptive sensory analysis, aromatic compound and consumer acceptance test will be combined with the palatability tests results at the conclusion of the studies.

### **Results and Discussion**

The initial aliquot of sorghum selected for the study was purchased from a local grower from 2013 crop year production. The material was milled at the Hal Ross Flour Mill on the Kansas State University campus (Manhattan, KS) in July of 2014. The millers were instructed to produce a good quality sorghum flour and to collect each of the secondary more fibrous product streams and the germ. In this case, what was originally anticipated as various layers of shorts, middlings and bran was most effectively captured as "mill-feed" (Table 1). This was the first sorghum milling activity at the new mill, so it was important to have an understanding of required facility settings and yields for this grain. The yield of flour at 69 percent was more than adequate to support the research feeding study. The yield of the mill-feed as a catch-all for the fibrous stream was more than adequate to move forward with the project, capturing a pure bran stream was not likely to be feasible for yield and mill capabilities. So, it was decided that the mill-feed would effectively serve the purpose for the intended project as it contained the bran of interest and some endosperm as well. Finally, there was every intention to collect sufficient amounts of germ to incorporate into research diets for the feeding study. But, the yield at less 1 percent was not going to allow adequate material to produce foods for this work. Thus, this treatment was abandoned for the feeding studies. However, samples will be evaluated for nutrient composition.

Additional evaluation in the lab were undertaken to work through some of the milling issues described above (Table 1). In the lab the sorghum was milled into the same three fractions and lost material was also quantified. The average yield of flour across four replications was just short of 50 percent. This is much lower than that noted for the initial HRFM test and can be explained by a less efficient roller milling system at the lab and the absence of a purifying system. The mill-feed fraction was relatively close (~35 percent) to that observed in the prior HRFM test; and the germ yield was much improved. But the lab data can differ substantially from actual production.

The whole sorghum starting material moisture was expected for stored grain and each fraction retained much of this moisture level (Table 2). The crude protein was slightly lower than what was expected for whole sorghum (9.55 percent), probably due to sampling error, but was within the expected range for the other fractions (from 10.80 percent in flour to 14.50 percent in the germ). The fat in the whole sorghum and flour were expected (2.94 and 2.32 percent). The balance of which was distributed to the mill-feed and germ (6.08 and 6.14 percent, respectively). It was anticipated that the germ would contain a higher proportion of crude fat and could suggest there is need to revisit the mill settings to better capture a greater proportion of the fatty germ from the bran. Finally, the ash content was distributed away from the flour and whole sorghum into the mill-feed and germ fractions, but the amount is not significant as it relates to companion animal nutritional considerations.

With this information in hand, a final milling activity for the purpose of producing quantities sufficient to produce diets was undertaken. The sorghum was purchased from a local grower from 2014 crop year production. It was milled at the HRFM in April 2015 with similar settings to that used previously. The yield results were similar to the first run at HFRM (Table 1), as each fraction yield had low standard deviations.

To further characterize the three sorghum fractions, proximate analyses were also done with the 2015 milled fractions (2014 crop-year), which measured similar results to the 2013 crop-year sorghum. Besides that, fiber analyses were conducted with the later milled fractions to have a better idea of the fiber component, since crude fiber is not an ideal method. Acid detergent (ADF) contents were 4 percent, 1.7 percent and 8.2 percent and neutral detergent (NDF) contents were 6.70 percent, 1.50 percent and 16.80 percent for whole sorghum, sorghum flour and mill-feed, respectively. The total dietary fiber (TDF) amount was 8.80% in whole sorghum, from which 2.60% was soluble and 6.20 percent), composed of 2.50 percent soluble fiber and only 0.70 percent insoluble fiber. Conversely, sorghum mill-feed had, as expected, the highest numerical quantity of TDF (20 percent), which consisted of 1.6 percent soluble fiber and 18.3 percent insoluble fiber. Moreover, sorghum mill-feed was the only fraction that had a detectable fraction of lignin of 2.90 percent. Total starch for mill-feed was numerically lower than that of whole sorghum and sorghum flour (43.8 percent vs 61.5 percent and 67.0 percent, respectively), and the presence of starch indicated that bran still had endosperm attached to it.

In the original work, it was proposed to use pigs as a model for dogs. However, due to scheduling issues and pig availability this become untenable. However, with a stroke of good fortune that was not anticipated during the preparation of the research proposal, dogs and facilities became available on campus. This marks a first for this type of research on the campus at Kansas State University and it cannot be stressed enough that it provides much, much higher value to the research and results to have the opportunity to evaluate sorghum in the target species.

The dietary treatments were based on formulas that we had previously used for other research projects (Table 4). The formulas are representative of adult maintenance foods and were intended to maximize the level of carbohydrate sources in order to more fully explore the effects of the various fractions. The control used a combination of brewers rice, corn, and wheat as the principal starch sources and the sorghum fractions replaced these in a near quantitative fashion for each of the respective treatments. The exception is that we worked diligently to balance the formulas so they were near iso-nutritional for all essential constituents. The remainder of the dietary ingredients consisted of a chicken byproduct meal and corn gluten meal as the primary protein sources. Beet pulp was used as a moderately fermentable fiber source, and chicken fat to supply the essential fatty acids. The remaining minerals and vitamins were included at levels sufficient for the diet to be nutritionally complete and balanced for dogs of all life stages. In addition to the nutritional constituents diets fed to dogs in the digestion study were produced with sufficient external markers (chromium sesquioxide and titanium dioxide) to allow their use in determining apparent total tract digestibility of each dietary treatment. For those experimental treatments produced for the sensory analysis and in-home feeding studies, the markers were omitted.

Following production of the diets, the nutrient composition was confirmed and in general terms met the expected levels predicted from the initial formulation (Table 5). Each of the experimental treatments had a moisture/dry matter level that was consistent with a typical dry extruded pet food. The crude protein was similar among the CON, WSD band FLD diets, but was somewhat higher for MFD. This should be expected based on the nutrient composition of the individual fractions observed above. The amount of fat on the product was somewhat controlled by the topical addition of fat. Despite this the control diet had a higher level of fat than each of the sorghum containing foods. The crude fiber composition of the diets was expected to be higher in the MFD dietary treatment, but due to analytical limitation this did not happen and it was consistent with crude fiber contents of sorghum fractions. Relative to commercial pet foods this would not be considered to be outside of typical. The macro- and trace-minerals were consistent across all treatments with formulated values and nutritional requirements.

The foods were produced using a single-screw extruder (Model X-20; Wenger Mfg., Sabetha, KS) in the extrusion laboratory at KSU (Tables 6-8). The flow rate during extrusion was intentionally set to be similar, but was lowest for MFD compared to the other treatments (average 150 vs 133 kg/h; Table 6). The product density exiting the extruder different slightly among the treatments; wherein, the CON was similar to the WSD and each was more dense (less expanded) than the FLD, but less dense (more expanded) than the MFD. A similar, but less pronounced relationship existed following drying; wherein, the CON and WSD were similar, WSD was also similar to FLD, and all were less dense than the FLD. Regardless of whether exiting the extruder or the dryer the kibbles containing the mill-feed were denser and less expanded. This is consistent with the lower starch level and higher fiber content of the mill-feed fraction.

The screw speed and preconditioner steam were consistent among treatments (Table 7). The preconditioner water was adjusted during production in an attempt to achieve consistent product physical parameters. However, the MFD received less Preconditioner water than CON, WSD, or FLD and the MFD was cooler at discharge than the other treatments. The extruder shaft speed was slightly faster for the MFD dietary treatment, and motor load was lower. Extruder steam was lowest for the MFD, intermediate for WSD and FLD and highest for the CON. But, for extruder water CON and MFD were set higher than WSD and FLD. Knife speed which controls the kibble length was not different among treatments. In general, the MFD differed in most extrusion parameters indicating some instability in the extrusion process.

The density measures observed above translated over to the expansion measures (Table 8). The piece diameter was largest for the FLD, followed by CON, then WSD and was smallest for MFD. The length was longest for WSD and MFD, lowest for FLD with CON similar to the extremes. Piece mass was lightest and piece volume was lowest for MFD compared to the other treatments. This translated in a low expansion for the MFD treatment. The sectional expansion ratio (SEI) of FLD was 1.92 times greater than MFD and the expansion of CON was 1.15 times that of WSD, with FLD being more expanded than CON and WSD being more expanded than MFD. Energy required to break the kibbles by 50 percent was similar among diets and averaged 144.3 N\*mm, whereas piece hardness was highest for FLD and MFD, with WSD having the lowest and similar hardness to CON and MFD. Although MFD had the highest mean, its variance was very high and that made it part of all the groupings. In general terms the CON, WSD, and FLD required similar process settings and produced products of similar physical size and density. The MFD on the other hand, was different than these products in most major process categories and suggests that the seed coat and fibrous nature of the mill-feed led to changes in product features. Whether this translates to a consumer issue, shelf-life concern, of product nutritional factor will need to be determined.

The 12 dogs were fed in a Latin Square designed experiment, which improves the number of replicates that can be used to evaluate the study and improve the strength of the statistics with so few animals. Further, each dog can serve as its own control. The dogs were intended to be fed a consistent nutritional quantity to support their weight for the duration of each period and the study. Food intake was computed for each dog at the beginning of each period based on their weight, estimates of the energy density of the food, and the use of empirical equations to predict food allowances. The amount of food given to the dogs was not different among the treatments in this study (average 186.8 g/d; Table 9). Feces were gathered each day by hand during the collection period, counted, scored, and weighted. The amount of wet feces excreted was in the order of MFD>WSD>CON>FLD; wherein MFD was nearly three times that of FLD (95.4 vs 32.6 g/d). The number of defecations per day was similar among the CON, WSD and FLD, and each fewer incidence than MFD. With more feces excreted daily and more defecations per day one may suspect a higher moisture level and perhaps softer stools. However, the MFD had the highest fecal score and differed from that of WSD and FLD.

Diet digestibility was estimated by four different methods: total collection, chromic oxide, titanium dioxide and acid insoluble ash. The data for the chromic oxide marker method is being presented (Table 10). This is the standard for determination of metabolizable energy determinations (AAFCO 2015) and has been the most common method used historically. By this method dry matter

digestibility followed a decreasing order (P<0.05) from FLD to CON, WSD and MFD, with a significant decrease of 15 percent units from WSD to MFD (86.0 percent, 83.0 percent, 81.1 percent and 65.9 percent). Organic matter, energy and crude protein digestibility followed similar relationships. Crude protein digestibility of the diets were somewhat lower than might otherwise be expected; wherein a more typical value would have been 80-84 percent. It was expected that the MFD dietary treatment would have a lower overall crude protein digestibility to that of the other treatments as this has been reported in other species previously. However, it was not proportionally lower than that observed for the dry matter, organic matter, or energy digestibility noted above. Given its high fiber content, the results of this study would suggest that it may have some other benefits to offer for energy restriction in obesity management and this should be explored. Further, diets using whole sorghum as a carbohydrate source have been reported to be similar to control diets in other studies, which was observed in the present study. As an additional positive, it appears that the sorghum flour dietary treatment (FLD) provided an improvement to digestion coefficients and might suggest new uses for "easy to digest" product placements.

The crude fiber digestibility, while interesting from a numbers perspective, doesn't necessarily have any basis in nutritional impact. The ash digestion has some impact as a gross indication of overall mineral utilization. In this instance it appears that the CON was more (P<0.05) digested than MFD and the WSD and FLD were intermediate. Calcium absorption is under the control of Vitamin D3, so this information is somewhat meaningless; interesting, but hard to interpret. In a similar manner so too is phosphorus. The potassium and sodium digestion, as electrolytes, seem to be better utilized in the CON, WSD and FLD diet and diminished for the MFD dietary treatment. The impediment for the MFD mineral (ash) utilization carries over to the rest of the trace elements as well. One might surmise that this could impede mineral fortification and mineral balance studies should be explored if this ingredient fraction is to be evaluated for full use at this level in the future.

One of the hypotheses of the study was that diets containing sorghum bran would provide higher antioxidant capacity for dogs due to its abundant phenolic acids content. The plasma ORAC value in dogs fed the MFD was more than two-fold higher (P < 0.05) than each of the other treatments CON, FLD and WSD (20,482 vs average 8,923  $\mu$ M TE/L). It was expected that the WSD would also lead to a higher antioxidant capacity, but this was not observed. The ORAC data is an important finding that can be used to strengthen the argument that sorghum has antioxidants that are beneficial for pets' health.

Based on this work, pet food companies should consider sorghum and its various fractions in their recipes. Sorghum bran is rich in fiber and phenolic compounds, and thus, could significantly contribute to diets that address obese or diabetic pets or those that have special conditions which might benefit from antioxidant fortification. Further, if starch digestibility is low part of it could escape to the colon and behave as fiber from resistant starch. This is associated with the prevention and control of diabetes, obesity and colon cancer (Birt, 2013). This phenomenon was not investigated in this study but should be evaluated in future research. As an additional benefit to milling, sorghum flour provided a slight improvement to digestion coefficients and might have application in "easy to digest" product placements. A future area of research would be to determine the optimum amount of sorghum bran to incorporate in a food to benefit health while retaining overall nutrient digestibility.

The trained sensory panel scored the MFD highest for "brown color" CON and FLD lowest, and the WSD intermediate (Table 11). This corresponds well with the color of the carbohydrates used to make the foods. The sensory scores are ranked on an intensity measure of 1-15 and the "brown color" scores were some of the highest recorded for appearance. The remainder of the appearance scores were quite low so as to be considered "slight." Despite some of the processing differences noted in the extrusion above, the panel did not detect differences in the porosity of the kibbles. The CON, WSD and FLD had a higher "oily" score than the MFD. The FLD had the lowest "grainy" score, WSD the highest with CON and MFD intermediate. The FLD treatment also had the lowest fibrous score with CON and WSD the highest and MFD similar and intermediate to each extreme. Surface roughness was similar for CON, WSD and MFD and each was greater than FLD. From these visual surface observations one can conclude that the sorghum flour based food was a smoother and more refined looking product, which is

consistent with a milled and refined flour compared to whole grains or grain fractions as would be seen with CON, WSD and MFD, respectively.

Regarding aroma scores (Table 12), there were few to any differences detected by the panel among the treatments, and all scores would be considered in the "slight" category in intensity. Barnyard, meaty, broth and liver were not affected by the dietary treatment. The toasted aroma was noted as differing between treatments with the highest values for the MFD relative to WSD and FLD and CON were intermediate and similar to each extreme. Whether this is a function of the ingredient mix or the physical food processing is difficult to discern. For the remainder of the aroma attributes: grain, brown, vitamin, oxidized oil, stale, cardboard, musty, dusty and earthy, there were no differences identified between treatments.

The flavor profiles (Table 13) were also low scores overall with only two attributes identified as differing from the other treatments (musty and dusty). That the flavor scores were all "slight" and few differences detected would suggest that the panelists are "tuned" or predisposed for a very broad category of food items with some very intense flavor palates. Further that these foods are served at room temperature and may not have fully developed their flavor profile in a manner consistent with typical human sensory panels could lead to the lower intensity scores than pet practitioners might expect. Especially considering that the same aroma profiles were observed as different when the flavor profiles were not (e.g., toasted). The highest intensity flavor scores were observed with the "bitter" attribute. There was some expectation that this would be both more intense and that the WSD and MFD containing sorghum pericarp would be noticeably astringent or bitter. This was not observed and suggests that the foods should perform well in a commercial setting. For the musty attribute, the MFD had the only recorded scores and was slight at 0.30; whereas, the other treatments CON, WSD and FLD were zero. The dusty attribute was similar between CON, WSD and FLD and lower (P<0.05) than the MFD treatment. This is a reasonable observation and should probably be of larger intensity given the bran layer is concentrated in the MFD and would carry with it any potential contamination from molds, dust, or other agricultural field debris.

Aftertaste (Table 14) intensity scores across the panel were in the "slight" category. The aftertaste "barnyard" description was the greatest intensity for the CON, WSD and MFD and each greater than FLD. No aftertaste differences were detected between treatments for liver. But, "brown" aftertaste was greatest for MFD, and lowest for CON and FLD with WSD intermediate and similar to each extreme. The grain, cardboard, bitter and sour aftertaste attributes were not different between treatments.

The texture scores (Table 15) were more intense than previous sensory evaluations with scores in the moderate category. But, few differences between treatments were noted. Scores for fracturability, hardness, initial crispness, cohesiveness (mass) and graininess were not different due to treatment. There was a trend (P=0.0557) for a difference in the "gritty" attribute with the highest score for CON, WSD with FLD intermediate and similar to each and the lowest score for MFD. Based on raw materials used in production this is a reasonable observation.

For the consumer panel, the overall liking scores (Table 16) had a higher intensity and were unequivocal to what was observed in the trained panel. For overall liking, the CON and WSD were preferred over the FLD and MFD (P<0.05; 6.44 and 6.59 vs 6.17 and 6.08, respectively). Parsing these observations into some key attributes the overall appearance liking followed the same ranking with CON and WSD preferred over FLD and MFD (6.60 and 6.67 vs 6.23 and 6.00, respectively). Color liking was greatest for CON and WSD, intermediate and similar to the extremes for FLD and lowest for MFD. Aroma liking was not affected by treatment.

These results can be summarized in some part by the partial least squares regression analysis (Graphic 1); wherein, the various treatments (CON, WSD, FLD and MFD) are shown as green dots. They are distributed where they intersect relative to the liking scores and an independent variable. The CON, WSD, and FLD treatments were relatively nondescript regarding their association. However, the MFD was associated with Brown appearance and toasted.

For the in-home use test where actual dogs were fed in owner homes as a single bowl feeding test there were no differences in percent of daily intake among the treatments (Table 17). Based on prior

experience of the authors and some of the observations by the trained sensory panel this was not expected. It clearly suggests that pets in the home are less discriminating than what we might think. Pet food professionals receive near constant feedback through customer service and sales channels that there are issues with food intake. Specifically one of leading complaints from pet owners is "won't eat" and the most common praises is "he likes it." These are very nondescript regarding context or cause an effect. But, they are real to the pet owner. Given the low intensity scores that we see from this work with trained sensory panelists, the consistency from the consumer panel, and the in-home food consumption data it would appear that other "signals" of liking are at play beyond the ingredient and process described in this work. This is a significant finding from this type of work. These inconsistencies should be evaluated in a more deliberate fashion to better describe the disparity between consumer feedback and research observations. Clearly there is a translation between the two that is missing and vital.

### Conclusions

The overarching goal of this work was to characterize the milling fractions from sorghum, which might be developed as a potential market expansion opportunity in the pet food industry. The bran and the germ of sorghum are certainly two of those elements that have the potential to provide some unique elements to animals (and humans) dietary regime. Our observations about sorghum milling were that the yield of a bran/mill-feed fraction is certainly viable and may need to be more refined for future work. The germ content is small and was not collected in sufficient quantities at this stage to evaluate. The only way that this fraction would be viable would require a substantial market volume for the refined flour and bran trades and then there might be sufficient germ to further evaluate. While small, this fraction holds great interest though for potential novel compounds that it might provide. Its small yield may allow for a high margin opportunities should unique compounds with high utility be identified.

At more than 60 percent of the dietary treatments, the MFD was expected to have an impact on processing and this was borne out by the extrusion results primarily seen as a reduced expansion. Despite this, we had no issues with dietary acceptance in the kennel dogs or the in-home tests. However, at these levels, the MFD led to a greater fecal output, more defecations per day and higher fecal scores relative to the other treatments. Consequently, digestibility of most major nutrient attributes was lower for the MFD dietary treatment. This diet clearly contained a higher degree of total dietary fiber, so one should expect these sorts of results. It would be worthwhile to evaluate the MFD in the diet at a slightly lower level in future work and to compare it to other common fiber sources to gain more perspective on its utility. On the other hand, a major benefit of the MFD was the increase in antioxidant capacity (ORAC value) in dog plasma, which can be used as a significantly positive marketing claim. At the other end of the spectrum the FLD performed very well in extrusion processing and overall nutrient digestibility and the sorghum flour might be a candidate for a specialty ingredient used in various pet food applications.

While some differences were noted by the trained sensory panel, the intensity scores were low (slight category) and did not discern any remarkable or alarming attributes. This is very meaningful. Further, the liking scores by the consumer panel ranked the foods with the MFD lowest, it was somewhat surprising that they lumped the FLD in this same category. It was expected, if anything, that the WSD would have been ranked with MFD. Further, that the in-home test consumption did not differ between treatments suggests that the differences while measurable, were all within a range that could be considered normal in the overall population of foods and households. Further, this suggests that each of the fractions was acceptable to pet-owners and their pets alike and that with some refinement the fractions could become a part of the cornucopia of ingredients in the formulators' toolbox to address new products in the pet aisle.

In conclusion, this work has demonstrated that sorghum fractions have the potential of producing new ingredients in pet food that would fuel and support its dynamic growth. Sorghum fractions can provide attributes and benefits to specific health conditions, which could be addressed to specialty markets or to therapeutic diets.

### **Literature Cited**

Asquith, T.N. and Butler, L.G. 1986. Interaction of condensed tannins with selected proteins. Phytochemistry, 25; 1591-1593.

Birt, D. F., T. Boylston, S. Hendrich, J. L. Jane, J. Hollis, L. Li, et al. 2013. Resistant starch: promise for improving human health. A Review. American Society for Nutrition. Adv. Nutrition, 4: 587-601.

Bouchard, G. F., and Sunvold, G. D. 2000. Effect of dietary carbohydrate source on postprandial plasma glucose and insulin concentration in cats. Pp 91-101 in Recent Advances in Canine and Feline Nutrition Volume III. Proc. 2000 Iams Nutrition Symposium. G. A. Reinhart and D. P. Carey, ed. Orange Frazer Press, Wilmington, OH.

Brannan, G.L., Setser, C.S., Kemp, K.E., Seib, P.A., and Roozeboom, K. 2001. Sensory characteristics of grain sorghum hybrids with potential use in human food. Cereal Chem 78(6):693-700.

Carciofi, A.C., Takakura, F.S., and Prada, F. 2004. Evaluation of starch sources for canine diets. Abstract. Alltech Biotechnology Conference. Lexington KY.

Di Donfrancesco, B., Koppel, K., and Chambers, E. IV. 2012. An initial lexicon for sensory properties of dry dog food. J. Sens. Stud. 27: 498-510.

Dykes, L., and Rooney, L.W. 2005. Sorghum and millet phenols and antioxidants. Cereal Foods World 52, 105-111.

Dicko, M.H., Gruppen, H., Traore, A.S., Voragen, A.G.J., and van Berkel, W.J.H.. 2006. Phenolic compounds and related enzymes as determinants of sorghum for food use. Biotechnol Mol Biol Rev 1:21-38.

FAOSTAT3, 2011. Food and Agriculture Organization of the United Nations. Accessed 4Aug2013. http://faostat3.fao.org/home/index.html

Food Chemistry 2009, book- p. 707. "Cereals- Milling"

Griffiths, D.W., 1985. The inhibition of digestive enzymes by polyphenolic compounds. Exp. Biol. Med., 199: 504-516.

Kobue-Lekalake, R.I., Taylor, J.R.N., and de Kock, H.L. 2012. Application of the dual-attribute timeintensity (DATI) sensory method to the temporal measurement of bitterness and astringency in sorghum. Int J Food Sci Tech 47:459-466.

Koppel, K., Adhikari, K., Di Donfrancesco, B. 2013. Volatile compounds in dry dog foods and their influence on sensory aromatic profile. Molecules 18: 2646-2662.

Murray, S. M., Fahey, Jr., G. C., Merchen, N. R., Sunvold G. D., and Reinhart G. A. 1999. Evaluation of selected high-starch flours as ingredients in canine diets. J. Anim. Sci. 77:2180-2186.

Murray, S. M., Flickinger E. A., Patil, A. R., Merchen, N. R., Brent, Jr., J. L., and Fahey, Jr G. C. 2001. In vitro fermentation characteristics of native and processed cereal grains and potato starch using ileal chyme from dogs. J. Anim. Sci. 79:435-444.

Rooney, L.W., and Serna-Saldivar. S.O. 2000. Sorghum. In Handbook of Cereal Science and Technology. 2nd Edition. Ed Karel Kulp and J.G. Ponte, Jr. Marcel Dekker, Inc. New York pp 149-175.

Sunvold, G.D., G.C. Fahey, Jr., N. R. Merchen, E.C. Titgemeyer, L.D. Bourquin, L.L. Bauer, and G.A. Reinhart. 1995. 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.

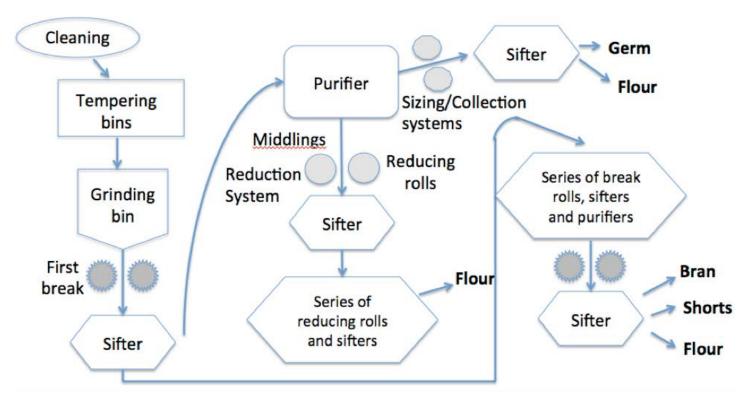
Sunvold, G. D., and Bouchard. G. F. 1998. The glycemic response to dietary starch. Pages 123-131 in Recent Advances in Canine and Feline Nutrition Volume II. Proc. 1998 Jams Nutrition Symposium. G. A. Reinhart and D. P Carey, ed. Orange Frazer Press, Wilmington, OH.

Twomey, L.N., Pethick, D.W. Rowe, J.B., Choct, M., Pluske, J.R., Brown, W., and Laviste, M.C. 2002. The use of sorghum and corn as alternatives to rice in dog foods. J. Nutr. 132:1704s-1705s.

Vazquez-Araujo, L., Chambers, E.IV., Cherdchu, P. 2012. Consumer input for developing human food products made with sorghum grain. J Food Sci 77(10):S384-S389.

## Appendices

Figure 1. Simplified milling diagram.



#### Figure 2. Flour mill settings used to produce sorghum fractions.

MDD0   1BK   2   800   31.50   250   350   4.5   11.3   D   18   2   0.2   52   .   1   1.2.5   .   350   1.0   15   0.458   0.0     MD00   284   2   800   31.50   250   450   5.7   14.6   D   18   4   0.2   PT5   W   1:2.5   .   350   7.5   10   0.313   0.0     MD0M   284   2   800   31.50   250   550   7.0   17.8   D   18   4   0.1   PT5   W   1:2.5   B   350   7.5   10   0.313   0.0     MD0M   484   2   800   31.50   250   650   8.3   21.0   A   69   4   0.1   PT5   W   1:2.5   B   350   7.5   10   0.313   0.0     MD0M   154/202   2   500   15.6   250   850   1.8   6   -   -   1:2.5   8   350   7.5	Rollermill     Grinding Rols       B	(uri			j.																							
n     n	B     B	(uri			-							Flowshe	et#:					Date:				Comments:						
B     B	B     B	(uni)	-	1 3			-	Flutes	_	_	_	_	_	_	-	Smooth roll	Feed rol	ls	_	-	_	Transmission	-	Drive	_			-
MDD0   28k   2   800   31.50   250   450   57   14.6   D   18   4   0.2   PT5   W   I:2.5   ·   350   ·   ·   0.01     MDDM   28K   2   800   31.50   250   450   57   14.6   D   18   4   0.2   PT5   W   I:2.5   B   350   7.5   10   0.313   0.01     MD0M   38K/D1   2   800   31.50   250   550   7.0   17.8   D   18   4   0.1   PT5   W   I:2.5   B   350   7.5   10   0.313   0.01     MD0M   48k   2   800   31.50   250   650   6.0   4   0.1   PT5   W   I:2.5   B   350   7.5   10   0.313   0.01     MD0M   58k   2   800   31.50   250   850   10.8   27.5   A   18   6   I   I:2.5   8   350   7.5   10   0.313 <td< th=""><th>MDD0     28K     2     8       MDDM     28K     2     8       MDDM     38K/D1     2     8       MDDM     58K     2     8       MDDM     58K     2     8       MDDM     58K     2     8       MDDM     154/D2     2     5       MDDM     2512/D3     2     5       MDD0     1M     2     8       MDD0     2M     2     5       MDD0     2M     2     8       MDD0     2M     2     8       MDD0     2M     2     8       MDD0     2M     2     8       MDDM     3M     2     5       MDUM     4M     2     5</th><th>Lengt</th><th>Length (rum</th><th>Ē</th><th></th><th>de 3</th><th>atercooling</th><th>Flutes per circum</th><th>5</th><th>s per</th><th>Grinding action</th><th>yle of</th><th>Spiral %</th><th>tand/mm</th><th>Land / in.</th><th>Tappering / µ (on diameter)</th><th>Type front</th><th>2</th><th>pag</th><th>200</th><th>Distribution Bridge</th><th>pet</th><th>Scrapper type</th><th></th><th>Mator / kW</th><th>~</th><th>N</th><th>HP / cwr.</th></td<>	MDD0     28K     2     8       MDDM     28K     2     8       MDDM     38K/D1     2     8       MDDM     58K     2     8       MDDM     58K     2     8       MDDM     58K     2     8       MDDM     154/D2     2     5       MDDM     2512/D3     2     5       MDD0     1M     2     8       MDD0     2M     2     5       MDD0     2M     2     8       MDD0     2M     2     8       MDD0     2M     2     8       MDD0     2M     2     8       MDDM     3M     2     5       MDUM     4M     2     5	Lengt	Length (rum	Ē		de 3	atercooling	Flutes per circum	5	s per	Grinding action	yle of	Spiral %	tand/mm	Land / in.	Tappering / µ (on diameter)	Type front	2	pag	200	Distribution Bridge	pet	Scrapper type		Mator / kW	~	N	HP / cwr.
MDD0   28k   2   800   31.50   250   450   57   14.6   D   18   4   0.2   PT5   W   I:2.5   ·   350   ·   ·   0.01     MDDM   28K   2   800   31.50   250   450   57   14.6   D   18   4   0.2   PT5   W   I:2.5   B   350   7.5   10   0.313   0.01     MD0M   38K/D1   2   800   31.50   250   550   7.0   17.8   D   18   4   0.1   PT5   W   I:2.5   B   350   7.5   10   0.313   0.01     MD0M   48k   2   800   31.50   250   650   6.0   4   0.1   PT5   W   I:2.5   B   350   7.5   10   0.313   0.01     MD0M   58k   2   800   31.50   250   850   10.8   27.5   A   18   6   I   I:2.5   8   350   7.5   10   0.313 <td< td=""><td>MDDM     28K     2     8       MDDM     35K/D1     2     8       MDDM     45K     2     8       MDDM     55K     2     8       MDDM     155/202     2     5       MDDM     155/203     2     5       MDD0     1M     2     8       MDD0     2M     2     8       MDD0     2M     2     8       MDD0     2M     2     8       MDDM     2M     2     5       MDDM     2M     2     5       MDDM     2M     2     5       MDDM     2M     2     5       MDDM     4M     2     5</td><td>800</td><td>800 3</td><td>1.50 25</td><td>0</td><td></td><td></td><td>350</td><td>4.5</td><td>11.3</td><td>D</td><td>18</td><td>2</td><td>0.2</td><td></td><td></td><td>52</td><td></td><td></td><td></td><td></td><td>1.2.5</td><td></td><td>350</td><td>11.0</td><td>15</td><td>0.458</td><td>0.03</td></td<>	MDDM     28K     2     8       MDDM     35K/D1     2     8       MDDM     45K     2     8       MDDM     55K     2     8       MDDM     155/202     2     5       MDDM     155/203     2     5       MDD0     1M     2     8       MDD0     2M     2     8       MDD0     2M     2     8       MDD0     2M     2     8       MDDM     2M     2     5       MDDM     2M     2     5       MDDM     2M     2     5       MDDM     2M     2     5       MDDM     4M     2     5	800	800 3	1.50 25	0			350	4.5	11.3	D	18	2	0.2			52					1.2.5		350	11.0	15	0.458	0.03
MODM   33K/D1   2   800   31.50   250   70   17.8   0   18   4   0.1   PT5   W   1   1:2.5   0   350   7.5   10   0.313   0.02     MDDM   48x   2   800   31.50   250   660   6.3   21.5   A   69   4   0.1   PT5   W   1:2.5   B   350   7.5   10   0.313   0.02     MDDM   58x   2.8   800   31.50   250   850   10.8   27.5   A   69   4   0.1   PT5   W   1:2.5   B   350   7.5   10   0.313   0.02     MDDM   152/D2   2   500   10.8   27.5   A   18   6   -   -   -   11.25   B   350   7.5   10   0.313   0.02     MDDM   152/D2   250   10.8   11.5   29.1   A   18   6   -   -   11.23   5   20   10   0.313   0.02   0.313	MDDM     30x/01     2     8       MDDM     40x     2     8       MDDM     50x     2     8       MDDM     51x/D2     2     5       MDDM     2xiz/D3     2     5       MDD0     1M     2     8       MDD0     2M     2     8       MDD0     2M     2     8       MDDM     2M     2     8       MDDM     3M     2     5       MDDM     2M     2     5       MDDM     2M     2     5       MDDM     2M     2     5       MDDM     2M     2     5       MDDM     4M     2     5												4			-							×.					
MDDM   43x   2   800   31.50   250   650   8.3   21.0   A   69   4   0.1   PT5   W   I   1:2.5   B   350   7.5   10   0.313   0.02     MDDM   59x   2   800   31.50   250   850   10.8   27.5   A   69   4   0.1   PT5   W   I   1:2.5   B   350   7.5   10   0.313   0.02     MDDM   15X/202   2   500   19.69   250   850   10.8   27.5   A   18   6   I   PT5   W   I   1:2.5   B   350   7.5   10   0.313   0.02     MDDM   15X/202   2   500   19.69   250   150   15   24.1   A   18   6   I   I   I   12.5   B   350   7.5   10   0.313   0.02     MDD   24   800   31.50   250   10.8   251.1   A   18   6   I   I   I </td <td>MDDM     43x     2     8       MDDM     53x     2     8       MDDM     1.512/02     2     51       MDDM     2.512/03     2     51       MDDO     1     2     51       MDDO     2     8     3       MDDO     2     8     3       MDDO     2     8     3       MDDM     2.21     2     8       MDDM     2.41     2     5       MDDM     4.44     2     5</td> <td>800</td> <td>800 3</td> <td>1.50 25</td> <td>0</td> <td></td> <td></td> <td>450</td> <td>5.7</td> <td>14.6</td> <td>D</td> <td>18</td> <td>4</td> <td>0.2</td> <td></td> <td></td> <td>PTS</td> <td>W</td> <td></td> <td></td> <td></td> <td>1:2.5</td> <td>B</td> <td>350</td> <td>7.5</td> <td>10</td> <td>0.313</td> <td>0.02</td>	MDDM     43x     2     8       MDDM     53x     2     8       MDDM     1.512/02     2     51       MDDM     2.512/03     2     51       MDDO     1     2     51       MDDO     2     8     3       MDDO     2     8     3       MDDO     2     8     3       MDDM     2.21     2     8       MDDM     2.41     2     5       MDDM     4.44     2     5	800	800 3	1.50 25	0			450	5.7	14.6	D	18	4	0.2			PTS	W				1:2.5	B	350	7.5	10	0.313	0.02
MDD 58 2 800 31.50 250 250 100 252 A 69 4 0.1 PT5 W V 1:2.5 B 350 7.5 10 0.313 0.02   MDDM 152/02 2 500 19.69 250 15.69 250 15.69 250 16.69 250 1.5 25 A 18 6 A A 1.6 A 1.6 A 1.12.5 8 350 7.5 10 0.313 0.02   MDD0 1.52/02 2 500 15.69 250 10.6 25.7 A 18 6 A A 1.6 A 1.6 A 1.12.3 5 20 7.5 10 0.313 0.02   MDD0 1M 2 800 31.50 250 A 1.8 6 A A 10 A 1.12.3 5 200 1.1.6 1.5 8.8 30.7 7.5 10 0.313 0.02   MDD0 1M 2 800 31.50 250 1.5 250 1.5 250 1.5 260 1.1.23 5 200 1.1.1.23	MDDM     59K     2     81       MDDM     1.512/02     2     51       MDDM     2.512/03     2     51       MDD0     1M     2     81       MDD0     1M     2     81       MDD0     2M     2     81       MDDM     2M     2     81       MDDM     3M     2     51       MDDM     3M     2     51       MDDM     3M     2     51       MDDM     4M     2     51	800	800 3	1.50 25	0			550	7.0	17.8	D	18	4	0.1			PT5	W				1:2.5	B	350	7.5	10	0.313	0.02
MDDM 15/2/02 2 500 19.69 250 10.8 27.5 A 18 6   MDDM 15/2/03 2 500 19.69 250 900 11.5 25.1 A 18 6   MDDM 25/2/03 2 500 19.69 250 900 11.5 25.1 A 18 6   MDD0 1M 2 800 31.50 250 5mooth 30-35 X 1:1.23 5 220 1.0 15 0.48 0.02   MDD0 2M 2 800 31.50 250 5mooth 30-35 X 1:1.23 5 220 1.0 15 0.48 0.02   MDDM 2M 2 500 19.69 250 5mooth 30-35 X 1:1.23 5 220 1.0 15 0.48 0.02   MDDM 2M 2 500 19.69 250 5mooth 30-35 X 1:1.23 5 220 1.0 0.03 0.02   MDDM 2M 2 500 19.69 250 5mooth 30-35 X 1:1.23 5 220 1.0 0.03<	MDDM     LSIZ/D2     2     5       MDDM     2SIZ/D3     2     SI       MDDO     IM     2     SI       MDDO     IM     2     SI       MDDO     IM     2     SI       MDDO     2M     2     SI       MDDM     2M     2     SI       MDIM     2M     2     SI       MUDM     3M     2     SI       MUDM     QU     2     SI       MUDM     4M     2     SI	800	800 3	1.50 25	0			650	8.3	21.0	A	69	4	0.1			PT5	W				1:2.5	B	350	7.5	10	0.313	0.02
MDD 2.5/2/3 2 500 19.69 250 900 11.5 29.1 A 18 6    1:2.5 B 350     MDD0 1M 2 800 31.50 250    30-35  X 1:1.23 5 220 1.0 15 0.458 0.03   MDD0 2M 2 800 31.50 250    30-35  X 1:1.23 5 220 1.0 1.5 0.458 0.03   MD00 2M 2 500 16.69 250    30-35 X 1:1.23 5 220 7.5 10 0.313 0.02   MD0M 2M 2 500 16.69 250    30-35 X 1:1.23 5 220 7.5 10 0.313 0.02   MD0M QU 2 500 16.69 250    30-35 X 1:1.23 S 220 7.5 10 0.314 0.02   MD0M <td< td=""><td>MDDM     2.5/2/D3     2     5/       MDDO     1M     2     8       MDDO     2M     2     8       MDDM     2M     2     8       MDDM     2M     2     8       MDDM     3M     2     5       MDDM     3M     2     5       MDDM     QU     2     5       MDDM     AM     2     5</td><td>800</td><td>800 3</td><td>1.50 25</td><td>0</td><td></td><td></td><td>850</td><td>10.8</td><td>27.5</td><td>A</td><td>69</td><td>4</td><td>0.1</td><td></td><td></td><td>PT5</td><td>w</td><td></td><td></td><td></td><td>1:2.5</td><td>В</td><td>350</td><td>7.5</td><td>10</td><td>0.313</td><td>0.02</td></td<>	MDDM     2.5/2/D3     2     5/       MDDO     1M     2     8       MDDO     2M     2     8       MDDM     2M     2     8       MDDM     2M     2     8       MDDM     3M     2     5       MDDM     3M     2     5       MDDM     QU     2     5       MDDM     AM     2     5	800	800 3	1.50 25	0			850	10.8	27.5	A	69	4	0.1			PT5	w				1:2.5	В	350	7.5	10	0.313	0.02
MDD0     M2     S00     31.50     250     smooth     30-35     X     1:1.23     S     220     1.1     1	MDD0     1M     2     8       MDD0     2M     2     8       MDDM     2M     2     5       MDDM     3M     2     55       MD0M     QU     2     55       MD0M     QU     2     55       MD0M     AM     2     55	500	500 1	9.69 25	0		-	850	10.8	27.5	٨	18	6		-			-	-		-	1:2.5	В	350	7.5	10	0.313	0.02
MDD0     24     2     800     31.50     250     smooth     30-35     X     1:1.23     5     200     -	MDD0     2M     2     88       MDDM     2M     2     55       MDDM     3M     2     56       MDDM     3M     2     56       MDDM     4M     2     56	500	500 1	9.69 25	0			900	11.5	29.1	A	18	6		_		-					1:2.5	B	350				
MDDM     2M     2     500     19,69     250     smeoth     30-35     X     1:1,23     S     220     7.5     10     0.313     0.02       MDDM     3M     2     500     19,69     250     smeoth     30-35     X     1:1,23     S     220     7.5     10     0.313     0.02       MDDM     QU     2     500     19,69     250     smeoth     30-35     X     1:1,23     S     220     7.5     10     0.313     0.02       MDDM     QU     2     500     19,69     250     smeoth     30-35     X     1:1,23     S     220     7.5     10     0.318     0.02       MDDM     4M     2     500     19,69     250     smeoth     30-35     X     1:1,23     S     220     7.5     10     0.318     0.02       MDDM     1T     2     500     19,69     250     smeoth     30-35     X     1:1,23     S<	MDDM     2M     2     Si       MDDM     3M     2     Si       MDDM     QU     2     Si       MDDM     QU     2     Si       MDDM     4M     2     Si	800	800 3	1.50 25	0					smo	oth					30 - 35					X	1:1.23	5	220	11.0	15	0.458	0.03
MDDM     3M     2     500     19,69     250     smeeth     30-35     X     1:1,23     S     220     -	MDDM     3M     2     50       MDDM     QU     2     50       MDDM     4M     2     50									smo	oth											1:1.23	5	220			-	
MD0M     QU     2     500     19.69     250     11.128     5     220     7.5     10     0.318     0.02       MD0M     4M     2     S00     19.69     250     1mooth     30-35     X     1:1.23     5     220     7.5     10     0.318     0.02       MD0M     4M     2     S00     19.69     250     1mooth     30-35     X     1:1.23     5     220     7.5     10     0.318     0.02       MD0M     5M     2     S00     19.69     250     1mooth     30-35     X     1:1.23     5     220     7.5     10     0.318     0.02       MD0M     SM     2     S00     19.69     250     1mooth     30-35     X     1:1.23     S     220     7.5     10     0.313     0.02	MDDM QU 2 50 MDDM 4M 2 50	500								smo	oth										х		5		7.5	10	0.313	0.02
MDDM     4M     2     500     19,69     250     smooth     30-35     X     1:1,23     S     220     Image: Constraint of the state of the	MDDM 4M 2 5									smo	oth										х		5	220			1	
MDOM     1T     2     500     19,69     250     smooth     30-35     X     1:1,23     S     220     7.5     10     0.313     0.02																							5		7.5	10	0.313	0.02
MDDM 5M 2 500 19.69 250 smooth 30-35 X 1:1.23 5 220 7.5 10 0.313 0.02	MDDM 1T 2 5	500			0					smo	oth					30 - 35					X	1:1.23	5	220				
										smo	oth										X		S					
Total - 3.42 0.27	MDDM SM 2 5	500	500 1	9.69 25	0					smo	oth					30 - 35					x	1:123	5	220	7.5	10	0.313	0.02
																							-	Total =			3.42	0.27

Figure 3. Extruder screw profile used to extrude the experimental diets control (CON), whole sorghum (WSD), flour (FLD) and mill-feed (MFD).

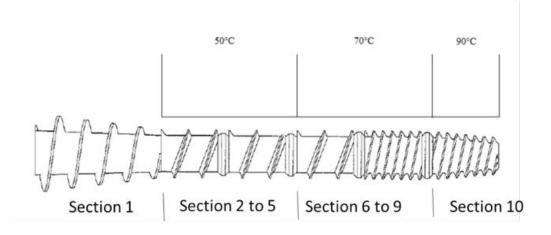
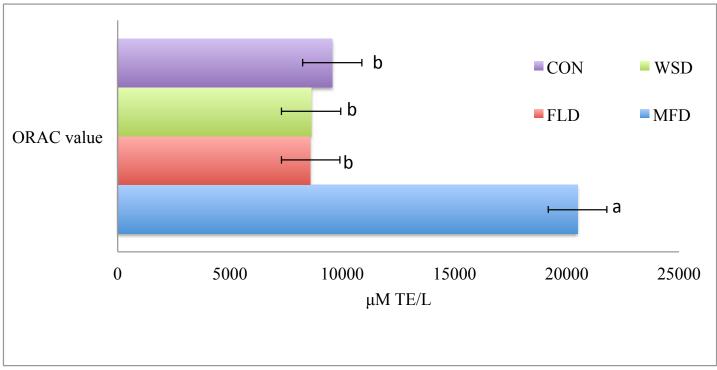


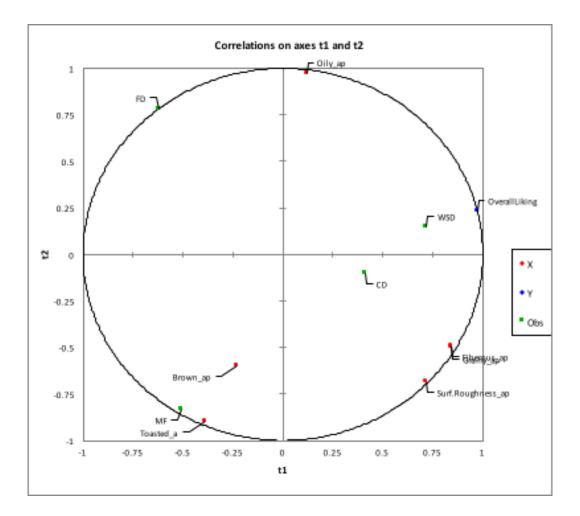
Figure 4. Oxygen radical absorbance capacity (ORAC) of plasma collected from dogs at the end of each period fed diets based on various sorghum fractions (N=12).



TE= Trolox equivalent.

<sup>ab</sup>Means within a row that lack a common superscript differ ( $P \le 0.05$ ).

Graphic 1. Partial Least Square Regression. Dependent variable (Y): Overall liking score (from Consumer panel), Explanatory variable (X): Descriptive sensory analysis (from human trained panel). Observations: Control (CON), whole sorghum (WSD), flour (FLD) and sorghum mill feed (MFD) containing diets.



## Table 1. Yield of red sorghum from 2014 (2013 crop year) and 2015 (2014 crop year) milled at the HRFM, and yield of red sorghum (2014 crop year) milled at a laboratory setting.

Milling location and year	Flour yield, %	Mill-feed yield, %	Germ yield, %	Loss, %
HRFM1	69.16 <u>+</u> 1.216	28.46 <u>+</u> 1.789	0.79 <u>+</u> 0.651	-
Laboratory2	49.97 <u>+</u> 3.487	38.50 <u>+</u> 8.109	2.93 <u>+</u> 0.955	8.59 <u>+</u> 5.838

1HRFM are expressed as the mean followed by SD of the years 2014 and 2015.

2Laboratory yields are expressed as the mean of 4 replications followed by their SD.

## Table 2. Nutrient analysis on as-is basis of red sorghum from the preliminary milling study (2013 crop-year sorghum purchased and milled at the Hal Ross Flour Mill; HRFM; July, 2014).

ltem	Whole Sorghum	Flour	Mill Feed	Germ
Moisture, %	13.66	12.36	11.27	11.86
Crude Protein, %	9.55	2.32	13.00	14.50
Crude Fat, %	2.94	6.08	6.08	6.14
Crude Fiber, %	0.97	n.d.	4.62	0.57
Ash, %1.30	1.30	1.61	2.56	2.56

## Table 3. Nutrient analysis on as-is basis of red sorghum used to incorporate into the dietary treatments (2014 crop-year sorghum milled on April/2015).

ltem	Whole Sorghum	Flour	Mill Feed
Moisture, %	12.94	12.37	11.49
Crude Protein, %	10.50	9.68	13.40
Crude Fat, %	2.81	3.68	3.68
Crude Fiber, %	1.18	n.d.	3.56
ADF, %	3.80	1.40	7.70
NDF, %	6.70	1.50	16.80
TDF, %8.80	8.80	3.20	20.0
Soluble Fiber, %	2.60	0.70	18.30
Insoluble Fiber, %	6.20	0.70	18.30
Lignin, %	n.d.	n.d.	2.90
Total Starch, %	61.5	67.0	43.8
Ash, %1.30	1.38	1.19	2.04

Table 4. Experimental diets to evaluate the effects of sorghum fractions on digestion in
dogs: Control (CON), whole sorghum (WSD), sorghum flour (FLD) and sorghum mill-feed
(MFD).

Ingredients, %	CON	WSD	FLD	MFD
Brewers rice	21.21	-	-	-
Corn	21.21	-	-	-
Wheat	21.21	-	-	-
Whole sorghum	-	64.69	-	-
Sorghum flour	-	-	62.31	-
Sorghum mill-feed	-	-	-	67.65
Chicken by-product meal	20.94	20.02	21.00	20.00
Chicken fat	5.34	5.52	5.52	3.29
Beet Pulp	4.00	4.00	4.00	4.00
Corn gluten meal	2.35	2.35	2.35	2.35
Calcium carbonate	0.75	0.35	0.23	0.67
Potassium chloride	0.49	0.52	0.64	0.19
Salt	0.46	0.45	0.46	0.43
Dicalcium phosphate	0.87	0.95	1.19	0.24
Choline chloride	0.20	0.20	0.20	0.20
Vitamin premixª	0.15	0.15	0.15	0.15
Trace mineral premix <sup>b</sup>	0.10	0.10	0.10	0.10
Natural antioxidant	0.07	0.07	1.21	0.08
Chromic oxide	0.25	0.25	0.25	0.25
Titanium dioxide	0.40	0.40	0.40	0.40
Ingredient total	100.00	100.00	100.00	100.00

<sup>a</sup>Vitamin premix: calcium carbonate, vitamin E supplement, niacin supplement, calcium pantothenate, vitamin A supplement, thiamine mononitrate, pyridoxine hydrochloride, riboflavin supplement, vitamin D3 supplement, biotin, vitamin B12 supplement, and folic acid.

<sup>b</sup>Trace mineral premix: calcium carbonate, zinc sulfate, ferrous sulfate, copper sulfate, manganous oxide, sodium selenite, and calcium iodate.

Nutrient	CON	WSD	FLD	MFD	SEM	P-value
Moisture, %	6.10	6.41	5.23	5.93	0.574	0.5717
Dry matter, %	93.9	93.6	94.8	93.8	0.65	0.6492
Organic matter, %	92.7	93.5	93.4	93.1	0.76	0.8937
Protein (crude), %	21.5a	21.4ab	21.2b	23.8a	0.43	0.0355
Fat (acid hydrolysis), %	12.10ª	10.70 <sup>ab</sup>	10.25 <sup>ab</sup>	9.48 <sup>b</sup>	0.392	0.0373
Fiber (crude), %	0.675 <sup>b</sup>	1.330 <sup>ab</sup>	0.725 <sup>b</sup>	2.710ª	0.3300	0.0346
Total starch, %	46.9ª	45.6ª	50.0ª	35.3 <sup>b</sup>	1.71	0.0140
Starch gelatinized, %	85.2 <sup>b</sup>	86.1 <sup>b</sup>	96.3ª	93.5ª	1.28	0.0084
Ash, %	7.24	6.52	6.59	6.86	0.744	0.8985
Calcium, %	1.64	1.56	1.40	1.44	0.081	0.2683
Phosphorus, %	0.885	0.965	0.880	0.850	0.0281	0.1535
Potassium, %	0.55	0.66	0.62	0.60	0.026	0.0845
Magnesium, %	0.095 <sup>b</sup>	0.135 <sup>ab</sup>	0.100 <sup>b</sup>	0.165ª	0.0083	0.0113
Sodium, %	0.290	0.290	0.275	0.250	0.0090	0.0936
Sulfur, %	0.295	0.295	0.265	0.290	0.0120	0.3572
Copper, ppm	19.2	17.0	15.8	18.3	2.10	0.7023
lron, ppm	179.0	180.5	161.5	187.0	7.06	0.2102
Manganese, ppm	27.8 <sup>ab</sup>	25.2 <sup>ab</sup>	19.6 <sup>b</sup>	34.0ª	2.24	0.0443
Zinc, ppm	143.5	159.5	136.5	165.5	15.55	0.5737

Table 5. Nutrient analysis of final experimental diets (as is) Control (CON), whole sorghum (WSD), sorghum flour (FLD) and sorghum mill-feed (MFD), fed to dogs during digestibility assessment and sensory analysis.

<sup>ab</sup>Means within a row that lack a common superscript differ (P  $\leq$  0.05).

# Table 6. Mean $\pm$ standard error of the mean (SEM) of process flow values measured during extrusion of experimental diets Control (CON), whole sorghum (WSD), sorghum flour (FLD) and sorghum mill-feed (MFD)

ltem	CON	WSD	FLD	MFD	P-value
Flow rate, kg/h	152a <u>+</u> 2.2	149a <u>+</u> 1.6	150a <u>+</u> 1.0	133b <u>+</u> 2.2	0.0071
Density OE*, g/L	325b <u>+</u> 2.2	333b <u>+</u> 3.8	286c <u>+</u> 1.4	425a <u>+</u> 19.5	0.0002
Density OD**, g/L	304bc <u>+</u> 2.7	317ab <u>+</u> 6.0	285c <u>+</u> 3.9	391a <u>+</u> 17.7	0.0098

\*OE= Kibbles collected out of the extruder; \*\*OD= Kibbles collected out of the dryer. abcMeans within a row that lack a common superscript differ (P < 0.05).

Table 7. Mean <u>+</u> standard error of the mean (SEM) of processing data collected during the production of dog diets by extrusion as controls (CON) or those containing whole sorghum (WSD), sorghum flour (FLD), or sorghum mill-feed (MFD).

ltem	CON	WSD	FLD	MFD	P-value
FS* speed, rpm	13.3	13.3	13.3	13.0	0.9436
PC** steam, kg/h	17.5 <u>+</u> 0.20	17.3 <u>+</u> 0.36	17.5 <u>+</u> 0.27	17.5 <u>+</u> 0.27	0.9611
PC** water, kg/h	15.8ª <u>+</u> 0.24	$15.6^{a} \pm 0.49$	$15.8^{a} \pm 0.57$	13.3 <sup>b</sup> ± 0.03	0.0012
PC** temp, °C	98.3 <sup>ab</sup> ± 0.46	98.5a <u>+</u> 0.21	$98.2^{ab} \pm 0.31$	97.1 <sup>b</sup> ± 0.12	0.0141
EX◆ shaft speed, rpm	322.7 <sup>b</sup> ± 3.57	319.7 <sup>b</sup> ± 0.80	319.4 <sup>b</sup> ± 0.82	383.7ª ± 0.21	<.0001
EX◆ steam, kg/h	19.33a ± 0.10	19.47a ± 0.21	5.34b <u>+</u> 0.47	3.19b ± 0.60	<.0001
Motor load, %	45.9 <u>+</u> 1.44	46.6 <u>+</u> 1.14	46.4 <u>+</u> 1.44	41.2 ± 0.99	0.0633
EX♦ water, kg/h	7.69 <sup>b</sup> ± 0.09	7.61 <sup>b</sup> ± 0.02	7.76 <sup>b</sup> ± 0.03	$10.17^{a} \pm 0.02$	<.0001
Knife speed, rpm	937 <u>+</u> 5.6	930 <u>+</u> 3.3	943 <u>+</u> 18.0	991 <u>+</u> 18.4	0.1071
SME•, kJ/kg	102.2 <u>+</u> 10.34	109.7 <u>+</u> 8.03	105.9 <u>+</u> 10.79	92.6 <u>+</u> 9.70	0.6278

\*FS= feed screw; \*\*PC = preconditioner;  $\bullet$ EX = extruder;  $\bullet$ SME= specific mechanical energy.

<sup>ab</sup> Means within a row that lack a common superscript differ ( $P \le 0.05$ ).

Table 8. Mean <u>+</u> standard error of the mean (SEM) of kibbles measurements and texture analysis of diets Control (CON), whole sorghum (WSD), sorghum flour (FLD) and sorghum mill-feed (MFD).

ltem	CON	WSD	FLD	MFD	P-value
Diameter, mm	14.2 <sup>b</sup> ± 0.08	13.2° ± 0.10	15.3ª ± 0.07	$11.0^{d} \pm 0.45$	0.0047
Length, mm	6.21 <sup>ab</sup> ± 0.213	6.94ª <u>+</u> 0.113	5.81 <sup>b</sup> ± 0.057	6.57ª <u>+</u> 0.052	0.0015
Mass, g/kib- ble	0.371ª ± 0.0110	0.357ª ± 0.0103	0.358ª ± 0.0036	0.288 <sup>b</sup> ± 0.0020	0.0002
Volume, cm3	0.981ª ± 0.0452	0.950ª ± 0.0300	$1.074^{a} \pm 0.0093$	0.634 <sup>b</sup> ± 0.0518	0.0038
Density, g/cm3	0.379 <sup>b</sup> ± 0.0064	0.376 <sup>b</sup> ± 0.0023	0.334° ± 0.0012	0.467ª ± 0.0400	0.0003
SEI*	$4.10^{b} \pm 0.048$	3.56° <u>+</u> 0.056	4.81ª ± 0.043	2.50 <sup>d</sup> ± 0.200	0.0002
Hardness, N	90.6 <sup>b</sup> ± 4.38	76.4 <sup>bc</sup> ± 3.85	120.5ª <u>+</u> 5.55	121.9 <sup>abc</sup> ± 19.17	0.0120
Energy for compression, N x mm	146 <u>+</u> 10.5	120 <u>+</u> 8.9	160 <u>+</u> 4.7	150.5 <u>+</u> 14.3	0.0742

\*SEI = Sectional expansion index.

<sup>abcd</sup> Means within a row that lack a common superscript differ ( $P \le 0.05$ ).

# Table 9. Food intake and excreted feces per day (on dry matter basis), number of defecations per day and fecal scores of dogs fed control (CON), whole sorghum (WSD), flour (FLD) diets and mill feed (MFD).

ltem	CON	WSD	FLD	MFD	SEM	P-value
Food intake, g/day	185	186	181	195	6.5	0.4818
Feces excreted, g/day	42.0°	55.7 <sup>b</sup>	32.6 <sup>d</sup>	95.4a	3.24	<.0001
Defecations per day	2.15 <sup>b</sup>	2.42 <sup>b</sup>	2.05 <sup>b</sup>	3.03a	0.14	<.0001
Fecal score	3.60 <sup>b</sup>	3.68 <sup>ab</sup>	3.78 <sup>ab</sup>	3.92a	0.068	0.0007

<sup>abcd</sup> Means within a row that lack a common superscript differ (P  $\leq$  0.05)

Table 10. Digestibility of control (CON), whole sorghum (WSD), flour (FLD) and sorghum
mill feed (MFD) containing diets by Beagle dogs (N=12) using chromic oxide as an external
marker.

Item, %	CON	WSD	FLD	MFD	SEM	P-value
Dry Matter	83.0 <sup>b</sup>	81.1°	86.0ª	65.9 <sup>d</sup>	0.44	<.0001
Organic Matter	88.0 <sup>b</sup>	85.8 <sup>b</sup>	90.7ª	70.6°	0.34	<.0001
Energy	87.2 <sup>b</sup>	85.4 <sup>b</sup>	90.3ª	70.2 <sup>c</sup>	0.68	<.0001
Crude Protein	77.5 <sup>b</sup>	76.3 <sup>b</sup>	81.8ª	67.2°	0.73	<.0001
Crude Fat	91.5ª	88.4 <sup>b</sup>	91.4ª	77.9°	0.37	<.0001
Crude Fiber	-75.2°	24.7ª	12.3ª	-29.7 <sup>b</sup>	7.25	<.0001
Ash	26.6ª	17.5 <sup>b</sup>	19.2 <sup>b</sup>	11.8°	1.78	<.0001
Calcium	-9.05 <sup>b</sup>	1.33ª	-5.74 <sup>b</sup>	-11.10 <sup>b</sup>	2.294	0.0023
Phosphorus	8.65 <sup>b</sup>	17.66 <sup>a</sup>	13.55 <sup>ba</sup>	-0.25 <sup>c</sup>	1.913	0.0010
Potassium	92.4 <sup>b</sup>	92.3 <sup>b</sup>	95.4ª	85.6 <sup>c</sup>	0.81	<.0001
Sodium	92.4b <sup>a</sup>	89.4 <sup>b</sup>	94.1ª	51.0°	1.19	<.0001
Magnesium	17.6ª	18.6ª	16.2ª	-7.3 <sup>b</sup>	2.165	<.0001
Iron	14.9ª	11.2ª	19.8ª	-24.8 <sup>b</sup>	3.22	<.0001
Copper	4.48 <sup>ba</sup>	9.37ª	1.32 <sup>b</sup>	-18.36°	2.797	<.0001
Zinc	-8.59 <sup>b</sup>	-1.91 <sup>ba</sup>	4.22ª	-20.36°	2.439	<.0001
Manganese	18.94ª	-6.68 <sup>b</sup>	-11.46 <sup>b</sup>	-27.86°	2.316	<.0001

<sup>abcd</sup> Means within a row that lack a common superscript differ (P  $\leq$  0.05).

## Table 11. Descriptive analysis for appearance of control (CON), whole sorghum (WSD), flour (FLD) and sorghum mill feed (MFD) containing diets with a trained human sensory panel.

ltem	CON	WSD	FLD	MFD	SEM	Р
Brown color	8.77ª	10.40 <sup>b</sup>	9.43ª	11.23°	0.2121	<.0001
Porous	2.13	2.33	2.13	2.50	0.1348	0.1578
Oily	2.0 <sup>ab</sup>	2.13ª	2.20ª	1.87 <sup>b</sup>	0.1100	<0.0001
Grainy	2.3 <sup>b</sup>	2.57ª	1.57°	2.17 <sup>b</sup>	0.1155	<0.0001
Fibrous	0.8ª	0.7ª	0.13 <sup>b</sup>	0.5 <sup>ab</sup>	0.1563	0.0201
Surface roughness	2.2ª	2.27ª	1.9 <sup>b</sup>	2.2ª	0.1564	0.0503

ltem	CON	WSD	FLD	MFD	SEM	P
Barnyard	2.4	2.4	2.3	2.73	0.2935	0.1561
Meaty	0.93	0.90	0.70	0.80	0.1729	0.6293
Broth	1.5	1.17	1.33	1.33	0.2310	0.2838
Liver	0.10	0.10	0.37	0.40	0.1555	0.2419
Toasted	1.43 <sup>ab</sup>	1.20 <sup>b</sup>	1.23 <sup>b</sup>	1.70ª	0.2581	0.0288
Grain	1.83	1.67	1.73	1.97	0.1642	0.5456
Brown	1.20	1.27	1.17	1.53	0.1359	0.2208
Vitamin	0.40	0.30	0.50	0.33	0.2190	0.6474
Oxidized Oil	0.80	0.37	0.50	0.70	0.2540	0.2208
Stale	0.43	0.20	0.37	0.27	0.2971	0.6103
Cardboard	1.93	1.73	1.80	1.87	0.1967	0.5732
Musty	0	0.07	0	0	0.0333	0.3997
Dusty	1.07	1.07	1.27	1.27	0.2996	0.4968
Earthy	0.10	0.07	0.07	0.07	0.1024	0.9783

Table 12. Descriptive analysis for aroma of control (CON), whole sorghum (WSD), flour (FLD) and sorghum mill feed (MFD) containing diets with a trained human sensory panel.

## Table 13. Descriptive analysis for flavor of control (CON), whole sorghum (WSD), flour (FLD) and sorghum mill feed (MFD) containing diets with a trained human sensory panel.

ltem	CON	WSD	FLD	MFD	SEM	Р
Barnyard	2.90	2.83	2.87	3.03	0.2218	0.7425
Meaty	1.30	1.37	1.03	1.37	0.2459	0.4366
Broth	1.97	1.83	1.67	1.97	0.1532	0.1480
Liver	1.2	1.2	1.03	1.23	0.3917	0.7934
Toasted	2.07	1.77	1.90	1.93	0.2849	0.4406
Grain	2.3	2.33	2.17	2.30	0.1933	0.7810
Brown	1.83	2.00	2.03	2.23	0.2609	0.1995
Vitamin	1.10	0.93	0.80	1.07	0.2944	0.3917
Oxidized Oil	1.27	1.17	1.03	1.27	0.2874	0.7721
Stale	0.47	0.30	0.27	0.60	0.2814	0.3853
Cardboard	2.27	2.07	2.17	2.23	0.1223	0.2790
Musty	Op	Op	Op	0.30ª	0.0817	0.0209
Dusty	1.17 <sup>b</sup>	1.40 <sup>b</sup>	1.33 <sup>b</sup>	1.70ª	0.3548	0.0063
Earthy	0.37	0.47	0.20	0.37	0.2423	0.4397
Sour	1.70	1.73	1.60	1.73	0.2809	0.7611
Salt	2.00	1.97	1.93	1.93	0.1962	0.9238
Bitter	3.70	3.93	3.67	3.93	0.3835	0.1728
Sweet	0.13	0.07	0.07	0.07	0.1032	0.8134
Metallic	0.37	0.50	0.30	0.77	0.3140	0.0946

Table 14. Descriptive analysis for aftertaste of control (CON), whole sorghum (WSD), flour
(FLD) and sorghum mill feed (MFD) containing diets with a trained human sensory panel.

ltem	CON	WSD	FLD	MFD	SEM	Р
Barnyard	2.90ª	2.63ª	2.20 <sup>b</sup>	2.87ª	0.2427	0.0023
Liver	1.23	1.13	0.80	0.93	0.3524	0.2504
Brown	1.10 <sup>b</sup>	1.30 <sup>ab</sup>	1.03 <sup>b</sup>	1.5 <sup>7a</sup>	0.2833	0.0282
Grain	1.53	1.77	1.73	1.60	0.2905	0.7277
Cardboard	2.03	1.80	1.80	2.00	0.1579	0.5706
Bitter	3.43	3.53	2.90	3.07	0.4378	0.1766
Sour	1.17	1.00	1.07	1.00	0.4940	0.6582

## Table 15. Descriptive analysis for texture of control (CON), whole sorghum (WSD), flour (FLD) and sorghum mill feed (MFD) containing diets with a trained human sensory panel.

Item	CON	WSD	FLD	MFD	SEM	Р
Fracturability	8.97	8.77	8.90	8.37	0.2575	0.0667
Hardness	8.70	8.67	9.07	8.63	0.2053	0.0330
Initial crispness	11.07	10.83	11.13	10.70	0.2150	0.1518
Cohesiveness (mass)	1.80	1.83	1.73	1.83	0.1041	0.8805
Graininess	8.30	8.33	8.37	8.00	0.3278	0.6733
Gritty	6.00ª	5.97ª	5.50 <sup>ab</sup>	5.37 <sup>b</sup>	0.3299	0.0557

## Table 16. Consumer panel (N=105 from 500 screened) of dog owners evaluation of "liking" (1-9 hedonic score; 1 dislike to 9 likes extremely) control (CON), whole sorghum (WSD), flour (FLD) and sorghum mill feed (MFD) containing diets with a trained human sensory panel.

Item	CON	WSD	FLD	MFD	SEM	Р
Overall Liking	6.44ª	6.59ª	6.17 <sup>b</sup>	6.08 <sup>b</sup>	0.1294	0.0003
Overall Appearance Liking	6.60ª	6.67ª	6.23 <sup>b</sup>	6.00 <sup>b</sup>	0.1342	<0.0001
Color Liking	6.46ª	6.59ª	6.34 <sup>ab</sup>	6.12 <sup>b</sup>	0.1434	0.0119
Aroma Liking	5.89	6.09	5.91	5.91	0.1416	0.4231

## Table 17. Dog panel (N=30 from 680 screened) of pet food samples acceptance by dogs (intake %) control (CON), whole sorghum (WSD), flour (FLD) and sorghum mill feed (MFD) containing diets with a one-bowl in-home palatability test.

ltem	CON	WSD	FLD	MFD	SEM	Р
Intake (%)	57.06	55.62	53.44	57.50	4.1044	0.1729