THE EFFECTS OF SORGHUM FRACTIONS ON PET FOOD EXTRUSION, DIGESTIBILITY AND ANTIOXIDANT CAPACITY IN DOGS

by

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Abstract

Novel ingredients fuel growth in the pet food market. Sorghum grain is a promising ingredient source. It grows in semi-arid regions and the pericarp is rich in phenolic compounds that are well-known antioxidants. The objectives were to determine the impact of milling sorghum on yield and composition of the various fractions, their impact on diet extrusion, and nutrient utilization when fed to dogs. Sorghum milling yields were 68.3% flour, 27.2% mill-feed, and 1.25% germ. Four nutritionally similar dog diets were extruded containing whole sorghum (WSD), sorghum flour (FLD), sorghum mill-feed (MFD), or a control diet (CON) with an equal proportion of corn, wheat and rice. The MFD had the highest ($P < 0.05$) bulk density and was 1.37-fold heavier than FLD. The FLD had a sectional expansion index (SEI) of 1.92 and 1.35-fold more than MFD and WSD. The FLD and MFD had the hardest kibbles ($P < 0.05$). Twelve Beagle dogs were fed the experimental diets in a 4 period replicated Latin square design with 9 d adaptation and 5 d total fecal collection (TFC). Fecal output was also estimated using acid insoluble ash (AIA), Cr_2O_3 and TiO_2. Plasma antioxidant activity was measured by oxygen radical absorbance capacity (ORAC) method. Fecal scores were highest ($P < 0.05$) for MFD treatment and lowest for CON, with FLD and WSD similar to both extremes. Dogs fed the MFD had the largest quantity ($P < 0.05$) of feces excreted and FLD the least. Dogs fed the FLD had highest ($P < 0.05$) overall nutrient digestibility values; whereas, MFD had the lowest values. TiO_2 estimates of fecal output correlated best to all other markers. The MFD had more than 2-fold ($P < 0.05$) the antioxidant value by ORAC versus the other treatments (20,482 vs average 8,923 μM Trolox Equivalent/L). This study suggests that sorghum flour would benefit easy-to-digest foods and the sorghum mill-feed could benefit foods needing indigestible fiber and antioxidants. Titanium dioxide may be a

better marker for fecal output than Cr_2O_3 or TFC. Future work should determine the optimal mill-feed level to provide health benefits without affecting nutrient digestibility.

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Chapter 1 - Literature review

Pet Food

The pet industry in the United States (US) is constantly growing with $58.04 billion of sales in 2014 and an estimated $60.59 billion in 2015 (APPA, 2015). Pet food comprises over one-third of sales, followed by veterinary care and medical supplies. The world's pet food market is also growing from an estimated $65.8 billion in 2010 to a forecasted $95.7 billion by 2017 (Taylor, 2012). Dogs and cats dominate the numbers. According to the 2015-2016 American Pet Products Association (APPA) National Pet Owners Survey, there were 77.8 million dogs and 85.8 million cats in the US, but cats lived in fewer households. Not to be overlooked, small mammals, excluding reptiles and fish, lived in 5.4 million households for a total of 12.4 million animals.

The pet food industry uses different processes to produce final products. Dry pet food comprises the largest portion of the US market, with around 70% of total pet food sales (Euromonitor, 2014). Other food forms include wet, semi-moist, soft-moist, jerkies, animal parts and injection-molded products. These require a number of common food production processes, such as extrusion, canning and baking, but often with a slight modification.

The largest proportion of dogs and cats are fed dry food in the US (9.2 billion US dollars in sales in 2014; Statista, 2016), and the greatest part of it is produced through extrusion. Food extruders are highly versatile processing units that form, shape, expand, restructure, cook, mix, texturize, and pasteurize a variety of food products (Riaz, 2000a). Selecting the optimal combination of ingredients and processing them under the appropriate conditions is essential for

consistent and stable pet food. Finding new ingredients to meet processing and market demands is an active area of exploration for the industry.

There is a growing trend for humanization of pet food around the world (Pet food Industry; PFI, 2015). As pets have shifted from living outdoors and serving utility functions to companions, and owners have shifted to "pet parents", the search for improved nutrition and selection of novel ingredients has created a drive for pet foods that fulfill human desires and, simultaneously, improve animal health. "Grain-free," "freeze-dried," and "refrigerated diets," among others have gained popularity as pet owners select foods similar to their own (Euromonitor, 2013a). Pet food market trends include an increased offering of high protein-low carbohydrate foods, limited or novel ingredient diets, natural non-synthetic preservative options, and species-specific ingredients. The hunt for new and novel ingredients continues to be aggressive. Sorghum is a gluten-free, non-genetically engineered (also known as non-GMO) grain, and is sustainable to grow in semi-arid environments (Lemlioglu-Austin, 2014). This makes it an interesting ingredient to explore and adapt to this dynamic market.

Sorghum

Sorghum is the fifth most important cereal crop in the world and is mostly used as food (55%) in Asia and Africa and as feed (33%) in the Americas (Reddy, 2010). Sorghum has the ability to adapt well to climate changes, particularly to drought, high temperature and soil salinity. There are more than 30,000 varieties of this cereal around the world. They are classified as sorghum, tannin sorghum, white sorghum and mixed sorghum according to US Grain Standards (USDA, 2008). The pericarp color is usually brown, but it can also be white, yellow, pink, orange, red or bronze. Sorghum can also be classified based on appearance and total extractable phenols; e.g., white sorghum with no detectable condensed tannins or anthocyanins

and little extractable phenols, red sorghum (red pericarp) rich in extractable phenols but little condensed tannins, black sorghum with high levels of anthocyanins, and brown sorghum with significant levels of condensed tannins (Awika et al., 2004).

In the United States sorghum represents the third largest cereal grain crop after wheat and corn. It is mostly fed to cattle and used to produce ethanol (Lemlioglu-Austin, 2014). Kansas and Texas are the states with largest sorghum production (Lemlioglu-Austin, 2014). However, this grain is still not fully explored in the growing pet food industry. The pet food market represents a large component of the agriculture and feed landscape with an estimated $23 billion in annualized sales in the US alone (Euromonitor, 2015). This translates into approximately 8.5 MMT (Euromonitor, 2013b); approximately 40% of that would come from grains produced in the US. The pet food industry is consumer marketing driven and in a constant search for new and alternative ingredients for which to promote their attributes in order to expand and differentiate products. Discovery of new ingredients is vital to foster continued growth and defensible claims are highly valued and rewarded in this high-margin market. Whole sorghum has already been explored in some pet food companies, but sorghum milled fractions could be new and exciting ingredient(s) if there was sufficient supporting information and instruction on their use.

The sorghum kernel is considered a naked caryopsis (Rooney and Miller, 1982). The caryopsis consists of three anatomical components: pericarp (outer layer), endosperm (storage tissue) and germ (embryo). All sorghums contain testa, which separates the pericarp from the endosperm (Fig 1.1). If the testa is pigmented sorghum will be red or brown and will contain anthocyanins. The endosperm is composed of the aleurone layer, peripheral, corneous and floury areas. The starch component, protein bodies and soluble fibers such as β-glucans and

hemicellulose are present in the corneous and floury endosperm (Serna-Saldivar and Rooney, 1995)

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%). Most of the starch is in the form of amylopectin (70-80%; Horan and Heider, 1946; Ring et al., 1982). The protein content and composition varies according to agronomic conditions; e.g., anything that decreases starch content, such as drought, increases sorghum protein (Waniska and Rooney, 2000). In a compilation of references summarized by Waniska and Rooney (2000), sorghum protein was found to be at or above 9%, which is slightly higher than corn. Lysine and threonine are the first and second limiting amino acids in the grain for monogastric animals. The fat content of sorghum is slightly lower (about 3%) than corn, and may be in part responsible for its lower metabolizable energy. The fatty acid linoleic acid (C18:2n6; essential for dogs and cats) comprises more than half the total fatty acids, and another third come from oleic acid (C18:1n9; not an essential fatty acid for pets). Less than 3% of the total fatty acids come from the omega-3 linolenic acid (C18:3n3). The fiber fraction is primarily insoluble (6.5 to 7.9% insoluble and 1.1 to 1.23% soluble β-glucans; Bach-Knudsen and Munck, 1985) with 86% of the insoluble fraction contained in the pericarp which provides protection for the kernel (Waniska and Rooney, 2000). Moraes et al. (2015) separated sorghum into bran and flour, and from these fractions the authors reported that bran contained the highest concentration of protein, lipids and total dietary fiber, followed by whole sorghum and finally decorticated sorghum flour, which was the lowest in all cited nutrients.

Sorghum could be an interesting ingredient for specialty markets. However, 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 nutritional value relative to corn. This reputation has been earned in some past studies that reported some sorghum varieties as having tannic acid (Armstrong et al., 1974; Kondos and Foale, 1983), which is a type of hydrolysable tannin. Some investigators found that tannic acid fed to rats and chickens negatively affected their health and performance (Armstrong et al., 1974; Glick and Joslyn, 1970a, b; Rayudu et al., 1970; Rostagno et al., 1973; Vohra et al., 1966), but it was never found in sorghum grains (Dykes and Rooney, 2006). Some sorghums, especially the pigmented types, contain some proportion of condensed tannins that may reduce feed efficiency due to hydrophobic and hydrogen bonding interactions with starch (Barros et al., 2012; Amoako and Awika, 2016), protein (Duodu et al. 2002, Scalbert et al., 2000), and chelation of some minerals (Scalbert et al., 2000). However, these effects were more pronounced *in vitro*. There is little known about these putative condensed tannin effects in different species *in vivo*. For animals like dogs and cats, whose diets are not based on a single starch source, the condensed tannins may not have a significant impact on their nutrition relative to other non-ruminant species that are fed a high percentage of a single grain.

Condensed tannins are more accurately called proanthocyanidins which are composed of a cauldron of different polyphenolic compounds produced as secondary metabolites by the plant that may support their natural defense system (Waniska et al, 1989). Some sorghum varieties have been selected to better exploit these benefits and several studies have been conducted that showed a strong correlation between tannins and phenolic compounds with antioxidant activity (Moraes et al., 2015; Dykes et al., 2005; Awika et al, 2003). The antioxidant activity of phenolic compounds found in sorghum is attributed to the radical scavenging ability of aromatic rings (Robbins, 2003). Radical scavenging ability refers to the property of antioxidants to inhibit the

oxidation of other compounds by reducing free radicals. Besides the antioxidant capacity of sorghum, it is also known to improve cardiovascular disease in humans due to the proanthocyanidins and other phenolic compounds that provide a cholesterol lowering effect (Lin et al., 1986; Tebib et al., 1997; Santos-Buelga and Scalbert, 2000) and blood thinning effect with red blood cell protection (Lee and Pan, 2003; Grinberg et al., 1997; Tedesco et al., 2000). There is also potential for sorghum in obesity treatment (Awika and Rooney, 2004). How best to take advantage of these benefits in a modern pet food becomes a vital question.

Sorghum milling

Most pet food producers look at sorghum as a whole ingredient only. However, we often deconstruct wheat into flour, corn into gluten, and soy into oil for incorporation into pet food. Thus, separation of sorghum into different fractions may hold promise. Dry milling of grain produces fractions, like flour and bran, for food utilization, while wet milling is used to produce alcohol, molasses and syrups (Rooney and Waniska, 2000). In the present study, all the milling cited will refer to dry sorghum milling. Dry milling operations include decortication and degermination, decortication alone, roller milling, and semimoist roller milling. Decortication and degermination occur when the grain is tempered and decorticated via abrasion, then tempered and degerminated by pin milling or impaction; lastly fractions are separated by sieving or gravity separation. Decortication alone occurs when sorghum is tempered and decorticated by abrasive mill or dehuller, usually when the grain is dry. For roller milling of sorghum the grain is tempered (approximately 16% moisture) and roller milled with wheat milling equipment. Finally, for semi-moist roller milling, sorghum is tempered to 30-35% moisture and milled using wheat flour rolls (Rooney and Waniska, 2000). Tempering the grain to 15-16% of moisture improves separation of flour and sometimes yields good products (Gomez, 1993).

Dehulling and decortication of grains is basically the removal of the pericarp through abrasion or roller milling before being milled into flour. Roller milling will reduce particle size and open the kernel by pressure and shear forces. Decortication reduces astringency, improves digestibility, and produces lighter colored products (Taylor and Dewar, 2001), which are positive attributes for consumers. When the sorghum kernel is opened, the pericarp, testa layers, and part of the germ is removed (Serna-Saldivar & Rooney, 1995) and the remaining kernel is reduced to flour. Grains, such as sorghum, can have their flour, germ, and bran separated according to particle size using sieves in a continuous flow gyratory sifter. Rollers are matched to the product needed: their size, surface flutes, rotation velocity, and 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 and collection systems. The grinding passages consist of breaks, sizing and middling, and the collection system includes quality and first tailing. Red sorghum bran is difficult to be removed without significant reduction of flour yield, whereas white sorghum has a better performance in roller milling (Rooney and Waniska, 2000; Awika et al., 2002).

Although sorghum flour may be preferred as a food source in human foods due to its sensory attributes, sorghum bran might become popular due to its health benefits resulting mostly from phenolic compounds present in the pericarp. Awika et al. (2005) found that the highest phenolic concentration occurred in the first fraction of decortication for most sorghum varieties; whereas, brown sorghum had its highest concentration of phenols in the second fraction. Thus, indicating that the testa layer had more phenols than the pericarp. The authors also concluded that decortication had a positive effect on lightness (sorghum color measured by colorimeter) and that tempering can significantly improve the efficiency of bran removal. Some

works have shown that an increased sorghum decortication time reduced total phenolic content of the final floury product (Awika et al., 2005; Dlamini et al., 2007; Chiremba et al., 2009; Buitimea-Cantua et al., 2013). Aboubacar et al. (2006) decorticated sorghum grain on an abrasive dehulling device to remove 10%, 20%, 30% and 40% of the outer layers of the kernel, and concluded that higher decortication levels led to increased starch and amylose contents. There are several products that can be obtained from a single crop through milling, and each can bring specific characteristics that may impact the food process and (or) provide desired attributes to the final consumer.

Sorghum in Extrusion

Extrusion is one of the most popular processes used to manufacture dog and cat food. It is accomplished through diet formulation (raw material characteristics and selection), hardware components (extruder barrel, extruder drive, etc.), and processing conditions (steam addition, temperature, etc.; Rokey, 2000). The ingredients are first ground into fine particles and mixed, and then they are conveyed to the extruder preconditioner. Preconditioning prior to extrusion will plasticize the raw materials with heat and moisture due to added steam and water, and contribute significantly to energy input, retention time and cook (Strahm, 2000). After exiting the preconditioner, the undeveloped dough is pumped through the extruder barrel. The barrel is divided into 3 zones: 1) the feeding zone where the material is raw with some added moisture; 2) the kneading zone that transforms the material into a dough-like mass from steam addition and mechanical energy, and 3) the final cooking zone where density is further increased and the combination of mechanical and thermal energies plasticize the material into a visco-amorphic flowing mass (Rokey, 2000). At the end of the extruder barrel molten dough is forced through a die opening to the outside ambient environment and expansion occurs.

Starch plays an essential role in dough formation and expansion. Kokini et al. (1992) described the expansion mechanism: 1) inside the extruder barrel the food material is converted into a viscoelastic melt with pressure, heat and water addition, 2) there is nucleation of bubbles within the starch polymer melt at sites where air was entrapped during the process, 3) these bubbles grow as the melt leaves the extruder die during the moisture flash-off, and 4) the high pressure inside the melt overcomes the mechanical resistance causing expansion. Starch gelatinization also occurs during extrusion cooking process. This consists of a disruption in the crystalline structure of the starch granule, absorption of water and subsequent swelling, which results in improved digestion by creating channels for enzymes like amylase to penetrate the glucose molecules (Lai and Kokini, 1991). Besides cooking, homogenizing and providing fluidity to the dough, water also acts as a plasticizer protecting starch granules from thermal and mechanical energies. Thus, low moisture content and high extrusion temperatures may lead to starch damage (Borries-Medrano et al., 2016). In contrast, the plasticizing effect of water in excess can reduce the material viscosity and the mechanical energy dissipation in the extruder, increasing product density and compressing bubble growth inside the dough (Ding et al., 2006). Bubbles formed inside the melt and temperature have a significant effect on extruded sample structure, expansion, and viscosity (Borries-Medrano et al., 2016). Thus having the right amount of starch, water, and optimal processing parameters are vital for a successful extrusion.

Pet food companies commonly use corn and rice as starch sources in their dry pet food recipes. Sorghum has a similar nutritional composition to corn, so it should behave similarly in extrusion. Dicko et al. (2006) found that the content of amylopectin from sorghum ranged from 45 to 54% on a fresh weight basis, which should significantly contribute to kibble expansion during extrusion. Amylopectin is composed of short chain α-(1,4) D-glucopyranose linkages

highly branched with α-(1,6) D-glucopyranose units (Blennow et al., 2001). Amylose is a linear starch molecule with α-(1,4) D-glucopyranose units. High amylopectin content leads to light, elastic, and homogeneous expanded textures, while a high amylose content leads to hard and less expanded extrudates (Moraru and Kokini, 2003). Dicko et al. (2006) reported a compilation of authors that found sorghum amylopectin content on a fresh matter basis to be from 45 to 55%, while amylose content ranged from 12 to 22%. Thus, whole sorghum should have good extrudability.

While starch is a structure forming ingredient, fiber impacts extrusion negatively and is considered a dispersed phase filler by the Guy Classification System (Guy, 2001). Sorghum bran is low in ash and protein, and rich in fiber (Kulamarva et al., 2009), so this fraction should not benefit the process. Moreover, it is known that gluten proteins play the main role in rheological properties of wheat dough formation (Faubian et al., 1990); whereas, gluten free cereals like sorghum make more fluid doughs (Kulamarva et al. 2009). This can negatively affect extrusion if not compensated by other functional components like starch. Turner (2004) reported that extrudates containing added sorghum bran resulted in decreased specific mechanical energy (SME) and expansion, and increased bulk density and breaking force due to the high fiber content.

Cooking processes like extrusion or baking promote several molecular physicochemical transformations and some authors have reported that cooking alters total phenolic content and (or) the proportion of oligomers and polymers in sorghum (Gupta & Haslam, 1978; Gu et al., 2004; Awika et al., 2003a). Cardoso et al. (2015) evaluated flavonoid profiles in sorghum samples extruded and processed in a dry heat oven and they found that extrusion diminished significantly the phenolic concentration. Awika et al. (2003a) quantified the procyanidin profile

of raw and extruded sorghum and found a significant increase in low polymerized tannins with a decrease in high polymerized tannins after processing. Thus, indicating there was cleavage of these compounds during extrusion. This may increase phenolic absorption by humans and animals. This effect was also observed in baking, but was more pronounced in extrusion (Awika et al., 2003a). Other effects from hydrothermal processing could have on phenolics include: release of bound phenolics from the food matrix, polymerization and oxidation of phenolics, complexation with macromolecules, thermal degradation, and maillard reactions (Taylor and Duodu, 2014). Insoluble complexes of sorghum kaifirins with polyphenols could also be formed during extrusion (Emmambux and Taylor, 2003). Kaifirins are proteins and part of the prolamin fraction located in the endosperm of sorghum, which are subdivided in α-, β- and τ-kaifirins. The latter kaifirin subtype has the highest molecular weight and is highly cross-linked with disulfide bonds. It becomes less digestible when cooked due to additional intramolecular cross-bonding (Serna-Saldivar and Rooney, 1995). Understanding the molecular transformations in extrusion and the parameters involved in the cooking process are essential to obtain the desired final product that should be digestible and nutritionally balanced for the target species.

Sorghum for dogs and cats

Pet food companies searching for novel sustainable ingredients to meet new product demands, along with the growing prominence of chronic or stress-related diseases have created an opportunity to explore alternative uses for sorghum. For example, specialty sorghums present a promising opportunity due to aspects like elevated antioxidant level and (or) fiber content (Awika et al., 2005). There is also evidence to suggest that certain fractions of sorghum may be beneficial to dog health as well. Several researchers have evaluated the use of whole sorghum in extruded dog and cat diets, but so far no work has been published regarding the use of sorghum

fractions in the pet food industry. 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 resulted in lower concentrations of glucose, longer time to peak glucose, and dampened insulin response curve after pets were fed a test meal containing sorghum 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. Moraes et al. (2015) found in an *in vitro* study that decorticated sorghum flour had the highest estimated glycemic index and sorghum bran had the lowest. Sorghum brans with high phenolic content and high antioxidant activity were shown to inhibit protein glycation in bovine albumin (that is the bonding of a protein with a sugar without an enzyme interaction; Farrar et al., 2008). Protein glycation negatively affects the function of biomolecules and it is an important process in the pathogenesis of diabetic complications. Thus, protein glycation inhibition is desired.

Sorghum condensed tannins and other phenolic compounds are beneficial in various metabolic processes due to their antioxidant activity. However, there is an offsetting drawback as some of the condensed tannin sub-fractions reduce protein digestibility of sorghum (Duodu et al., 2002). Proanthocyanidins precipitate proteins by formation of hydrogen bonds between phenolic residues and protein polar groups, digestive enzymes inhibition at a certain degree (amylase inhibitor, trypsin inhibitor), and trace mineral chelation, especially iron (Scalbert et al., 2000). 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 rates and that the astringent sensation lasts longer than does bitterness. Higher molecular weight phenolic compounds were strongly related to these attributes (Kobue-Lekalake et al., 2012). It is this aspect that may influence animal acceptability of foods manufactured with sorghums.

Sorghum is a gluten-free grain. Although gluten-free products are gaining popularity in human food, gluten intolerance is very rare in dogs. Irish setters were used as models to study celiac disease in humans (Marieta and Murray, 2012). These authors suggested that this breed can develop a purely innate (nonadaptative) response to gluten resembling what occurs in children with celiac disease (CD). Irish setters were the only dogs reported to have gluten intolerance, thus this is not a concern for dogs in general. However, pet food companies could use the gluten-free argument as a marketing tool, since there is a tendency for incorporating human nutrition trends into pet nutrition, even when there is no scientific basis.

Phenolic Acids and Antioxidant activity

There are two major categories of phenolics in sorghum: phenolic acids that are mostly benzoic or cinnamic acid derivatives (Fig 1.2; Hahn et al., 1983; Waniska et al., 1989) and flavonoids, which include procyanidins (tannins) and anthocyanins. Both genetics and environment affect phenolic composition of sorghum varieties (Awika, 2004). Phenolic acids are located in the pericarp, testa, aleurone layer and endosperm of sorghums and millets (Hahn et al., 1983; McDonough et al., 1986) and they can be found in free and bound forms (Dykes and Rooney, 2006). Bound phenolics represent the majority of total phenolics in sorghum and millets with 24-47% of the total being ferulic acid (Pasha et al., 2014). The highest concentration of phenolic compounds in sorghum were reported in the bran fraction; wherein, these phenolics

averaged 3.5 (Awika et al., 2005) and 3.8 (Moraes et al., 2015) times higher than in whole sorghum.

Anthocyanins confer colors to the sorghum varieties. Tannins in sorghum are mainly in the condensed form and are known as proanthocyanidins or procyanidins. They consist of polymerized flavan-3-ol and (or) flavan-3,4-diol units that form high-molecular weight polyphenols (Dykes and Rooney, 2006). The proanthocyanidins usually have (-)-epicatechin as extension units and catechin as terminal units (Gu et al., 2002, 2003; Gupta and Haslam, 1978). Tannins were shown to have a strong antioxidant activity in vitro (Hagerman et al., 1998), but the lack of standards make them difficult to be quantified.

Phenolic compounds behave as antioxidants due to the reactivity of the aromatic rings that are believed to be radical scavenging via hydrogen atom donation (Robbins, 2003), forming less reactive phenoxyl radicals with reactive oxygen species. Phenolic acids have been shown to inhibit cyclooxygenase-2 and to prevent colon cancer cells in humans (Karlsson et al., 2005; Gao et al, 2006). Koldas et al. (2014) found that phenolic compounds have antioxidant activity in lower concentrations and anticancer activity at higher concentrations in cells. Ferulic and p-coumaric are the most abundant phenolic acids in red sorghum (Hahn et al., 1983), and protocatechuic, caffeic, vanillic, p-hydroxibenzoic, gallic, and cinammic acid have also been identified (Svensson et al., 2010; Hahn et al., 1983). Svensson et al. (2010) found protocatechuic acid to be the most abundant phenolic acid in a red sorghum variety, while Hahn et al. (1983) found that ferulic, p-coumaric and protocatechuic acids were the most abundant bound phenolic acids and vanillic and ferulic acids were the most abundant free phenolic acids in two red sorghum selections. Bound forms of phenolic acids are much more concentrated than the free forms.

Condensed tannins in sorghum have been correlated with strong antioxidant potential in vitro (Hagerman et al., 1998; Riedl and Hagerman, 2001). Lolito et al. (2000) found that this is mainly due to oligomer chain length. Hahn et al. (1983) observed that fungal resistance did not correlate well with phenolic acids and in one of the red sorghum varieties it could be in part explained by its testa layer that was rich in tannins. However, a strong correlation between antioxidant activity and levels of ferulic acid in grains has been reported (Adom and Liu, 2002). Hydroxycinnamic acids like ferulic, caffeic, syringic and p-coummaric acids have been shown to have high antioxidant activities (Kim et al. 2006) because these molecules have the propiolic acid group (CH=CH-COOH) that confer higher antioxidant potential compared to the carboxyl group (COOH) in hydroxybenzoic acids (Cuvelier et al., 1992). Emmons et al. (1999) concluded that total antioxidant activity is a result of a complex mixture of several antioxidants and pro-oxidants. To date, no study has analyzed antioxidant capacity or phenolic acids concentration in dog plasma after sorghum consumption.

Several *in vitro* and *in vivo* studies have found that cereal grain phytochemicals may improve the antioxidant capacity in biological systems and there are several techniques available to explore this potential. Awika et al. (2003b) tried to establish a quick and suitable method to estimate antioxidant activity in sorghum and sorghum products in vitro. For that, the authors analyzed three commonly used methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) and oxygen-radical absorbance capacity (ORAC) and found that both DPPH and ABTS correlated highly with ORAC. Although the ABTS method was shown to be more suitable for sorghum, ORAC is the "only standardized *in vitro* method that uses biologically relevant free radicals" Awika (2003b). Awika (2003b) measured the ORAC value of black sorghum and brown sorghum brans (1,010 and 2,400-3,100 μmol TE/g,

respectively) which were significantly higher than blueberries μmol TE/g (87-870, Moyer et al., 2002). These are widely known to be good sources of antioxidants. Moreover, sorghum varieties with pigmented testa have the highest levels of phenols and antioxidant activity (Dykes et al., 2005).

The ORAC assay was developed by Cao et al. (1993). It measured the ability of an antioxidant to protect a target protein β-phycoerythrin from free radicals originating from peroxyl radical (ROO^-), hydroxyl radical (OH^-) and Cu^{2+} which then measured the extent of damage through the protein's loss of fluorescence. The standard radical was later chosen to be ROO^- since it is more common in biological systems (Cao and Prior, 2001). Ou et al. (2001) adopted fluorescein as the new target protein, as β-phycoerythrin led to results with poor repeatability due to interactions with other molecules. The ORAC method has the following mechanism: a peroxyl radical (ROO^-) is formed from the breakdown of 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), which can oxidize fluorescein and generate a product without fluorescence. Antioxidants suppress this reaction by a hydrogen atom transfer mechanism, so the concentration of antioxidant is proportional to the fluorescence intensity and it is assessed by comparing the net area under the curve to that of Trolox, a vitamin E analogue. Liyana-Pathirana and Shahidi (2006) found that ORAC values of wheat extracts were higher in bran, followed by whole grain and flour. In fact, bran had an ORAC value 37 times higher than flour. Phenolic acids are highly concentrated in the bran fraction and the bound forms contributed greatly to the results because they are more abundant.

Digestibility

When a new pet food is being developed it is common to evaluate diet digestibility in the target species to validate nutrition and safety of the product. Broadly, this provides information

about intake or acceptability, fecal output, stool consistency, nutrient utilization, and overall healthfulness. The total fecal collection method (Lindahl, 1963) is the gold standard to calculate apparent digestibility in many animal species. This method consists of collecting all feces and weighing all food consumed in a given period of time. The nutrients whose digestibility are to be determined are measured in both feces and food and apparent digestibility is calculated using equation 1.1:

$$\textit{Digestibility of nutrient} = \frac{\textit{(\%nutrient} \times \textit{food intake) - (\%nutrient} \times \textit{feces excreted)}}{\textit{(\%nutrient} \times \textit{food intake)}} \quad (1.1)$$

However, total fecal collection is not always possible and may be difficult to conduct with a high degree of accuracy. Total fecal collection is time consuming and requires meticulous planning, labor and full-time confinement of the animals. In wild species or those animals that are free-ranging it is impractical. An alternative to total fecal collection is the use of markers to estimate fecal output. A substance can be used as a marker for digestibility if it is unabsorbed and undigested by the animal, if it mixes homogenously with the digesta through the gut, and if it has no effect on the digestion metabolism of other nutrients (Schneider and Flatt, 1977). A common indicator used to calculate digestibility is chromium sesquioxide (Cr_2O_3; McCarthy, 1974) and it is to date the official indicator method for dogs and cats specified by AAFCO (2016) for determination of metabolizable energy. However, alternative methods are needed because the results obtained from using Cr_2O_3 can be variable, as the recovery rates for Cr_2O_3 have been reported to be between 75 to 87% (Moore 1957; Ishikawa 1966; Ishikawa and Sugimura 1973). Furthermore, it is difficult to obtain consistent repeatability among laboratories, and chromic oxide may be hazardous (Sales and Janssens, 2003). The determination of chromic oxide in feces is measured by atomic absorption spectrophotometry (Williams et al., 1962) which is a spectroanalytical procedure that quantifies chemical elements using the absorption of optical

radiation by free atoms in the gaseous state. Apparent digestibility using Cr_2O_3 can be calculated using equation 1.2 (AAFCO, 2016).

$$Digestibility\ of\ nutrient = \left[1 - \frac{(\%Cr_2O_3\ in\ food \times \%nutrient\ in\ feces)}{(\%Cr_2O_3\ in\ feces \times \%nutrient\ in\ food)}\right] \times 100 \quad (1.2)$$

Another substance that can be used as an external marker is titanium dioxide (TiO_2). It has the advantage over Cr_2O_3 of being a food color additive that is incorporated in food up to 1% (Code of Federal Regulations; 2015), so there are fewer concerns regarding animal safety. Plus, TiO_2 has the practicality for use in non-research animals because of its relative safety. For example, Hagen-Plantinga et al. (2014) used TiO_2 to assess the effect of age, body weight, sex and neutered status of privately owned dogs on energy digestibility, and found no significant differences between these physiological states. Titanium dioxide is a marker commonly used to determine digestibility in swine, chicken and cattle, and less common in dogs. A study conducted with broilers found TiO_2 recovery over 85% and DM digestibility was slightly lower compared to total fecal collection (Smeets et al., 2015). Titgemeyer et al. (2001) conducted digestibility studies with steers using TiO_2 as a marker and reported fecal recovery from 90 to 95%. Childs-Sanford and Angel (2006) used titanium dioxide to calculate intestinal transit time in dogs and maned wolves, and chromic oxide to estimate digestibility in these two species. Transit time did not differ between species or diets, only nutrient digestibility was slightly lower in maned wolves. If TiO_2 can be used as a marker for intestinal transit, it most probably could be a viable marker for digestibility.

To be most effective markers like Cr_2O_3 and TiO_2 need to be provided in a constant quantity. Addition to the diet fulfills this need. An interesting option to avoid the extra effort associated with adding a marker is to exploit something already present in the food. Acid insoluble ash (AIA) is an intrinsic mineral material found in food that is not digested or absorbed

by the animal gut. This characteristic allows it to be used as a marker. There have been several reports measuring AIA to calculate fecal output by ruminants, birds, rabbits, fish and pigs; but, very few with dogs. For example, McCarthy et al. (1974) verified that the 4N-HCl insoluble ash method was superior for calculating digestible energy (DE) and digestible nitrogen (DN) in pigs when compared to chromic oxide. They came to this conclusion because AIA yielded results that were more similar to estimates from total collection than those derived from Cr_2O_3. Of the two indicator methods both were similar for determining DE and DN. In another study, McCarthy et al. (1977) confirmed that grab samples (collected directly from the rectum) were as effective as the analysis of an aliquot of total feces voided. This would suggest intermittent sampling might be an acceptable benefit for using markers. They concluded that 4N HCl digestion to determine insoluble ash may be a viable method to estimate digestibility in pigs. Likewise, Vogtmann et al. (1975) concluded that AIA method lead to similar results to total fecal collection when determining ME and fatty acid digestibility in broilers. Atkinson et al. (1984) found a strong correlation in trout between digestibility calculated using Cr_2O_3 and AIA as markers. In a study conducted in Brazil with 5 dogs, apparent digestibility using Cr_2O_3 and AIA were highly correlated with total fecal collection method, and prompted the authors to conclude both markers were suitable options (Lobo-Junior et al., 2001). However, caution must be used when determining AIA in feedstuffs with low ash levels (e.g., Alfalfa; Keulen and Young, 1977). Fortunately, most pet food formulas contain more than sufficient ash to be effective.

The values obtained by the AIA digestibility method are often lower than those obtained by total collection by a small amount (McCarthy et al., 1974; McCarthy et al., 1977; Vogtmann et al., 1975; Lobo-Junior et al., 2001). This may be due to a failure in collecting all feces for the total collection method. However, there are also studies in which AIA overestimated apparent

digestibility when compared to other methods (Stein et al., 2006; Zanatta et al., 2013). Zanatta et al. (2013) found that both crude fiber and AIA were adequate markers to predict digestibility in dogs that were fed a soybean meal based diet, but AIA overestimated the digestibility of a poultry meal based diet. According to these authors it may be explained by an incomplete solubility of HCl soluble minerals like calcium and phosphorous that are abundant in bone. Vogtmann et al. (1975) developed the AIA procedure using 4N HCl in which a 10-gram sample was boiled for 30 minutes, and then ashed after the acid treatment. Later, Keulen and Young (1977) compared total fecal collection method with three laboratory analytical procedures for AIA in feed and feces, in which concentrated HCl, 4N HCl and 2N HCl, along with ashing sequence and ashing temperature were varied. The concentrated HCl and 4N HCl procedures both had one ashing after acid boiling, while 2N HCl had one ashing before and one after the acid treatment. The authors found that the dry matter digestibility determined by all AIA procedures and total collection method were statistically equal, but the 2N HCl was preferred because it was less time consuming and safer (since it used a lower acid normality and the samples were ashed before being boiled in acid, and eliminated the strong odor). They also reported that there were no significant diurnal AIA excretion patterns detected, so the time of the day feces were collected was not important. Sales and Janssen (2003) also concluded later that the 2N HCl procedure described by Keulen and Young (1977) was most effective, because it didn't overestimate nutrient digestibility from the ashing before acid boiling and thereby avoided erroneous accounting for HCl soluble minerals. Digestibility with AIA as an intrinsic marker is calculated using equation (1.3).

$$\text{Digestibility of nutrient} = \left[1 - \frac{(\%\text{AIA in food} \times \%\text{nutrient in feces})}{(\%\text{AIA in feces} \times \%\text{nutrient in food})}\right] \times 100 \qquad (1.3)$$

Processing of sorghum grain such as pressure-cooking, steaming, flaking, puffing or micronization increases the digestibility of starch due to the starch granule gelatinization and release from the protein matrix, facilitating enzymatic digestion (Harbers, 1975; McNeil et al., 1975). The total tract apparent nutrient digestibility for maize and sorghum were shown to be reduced for foods with greater mean geometric diameter and less starch gelatinization (Bazolli et al., 2007). In the same study, the authors found a linear reduction in nutrient digestibility with increasing cereal particle size for sorghum. There are several factors that influence nutrient digestibility and having an appropriate and reproducible marker to calculate fecal output is essential.

Summary

The pet food market in the US and worldwide is constantly growing and there is need to explore alternative ingredients. Sorghum is an important crop with interesting features such as "non-GMO", gluten-free, and it is rich in phenolic compounds that are concentrated in the pericarp and may confer antioxidant activity. Further, sorghum grows in semi-arid regions and has the ability to adapt well to climate changes. Some pet food companies have incorporated whole sorghum into their diets, but no companies currently use or promote sorghum fractions in their products. These may improve food processing and (or) animal health. Hence, there is need to evaluate the milling process on sorghum and how these sub-fractions of the seed might process and be accepted by pets. This work has to date not been addressed and is a glaring gap in knowledge. Therefore, the objectives are to determine the milling performance of a locally grown red sorghum, the effects the resulting sorghum fractions have on in the production of a complete extruded dog food, and to determine the acceptability, fecal scores, fecal output, and

antioxidant capacity when dogs are fed diets produced from these foods. The hypothesis is that sorghum milling fractions in a dog diet will provide potential for new ingredients beneficial for pet foods.

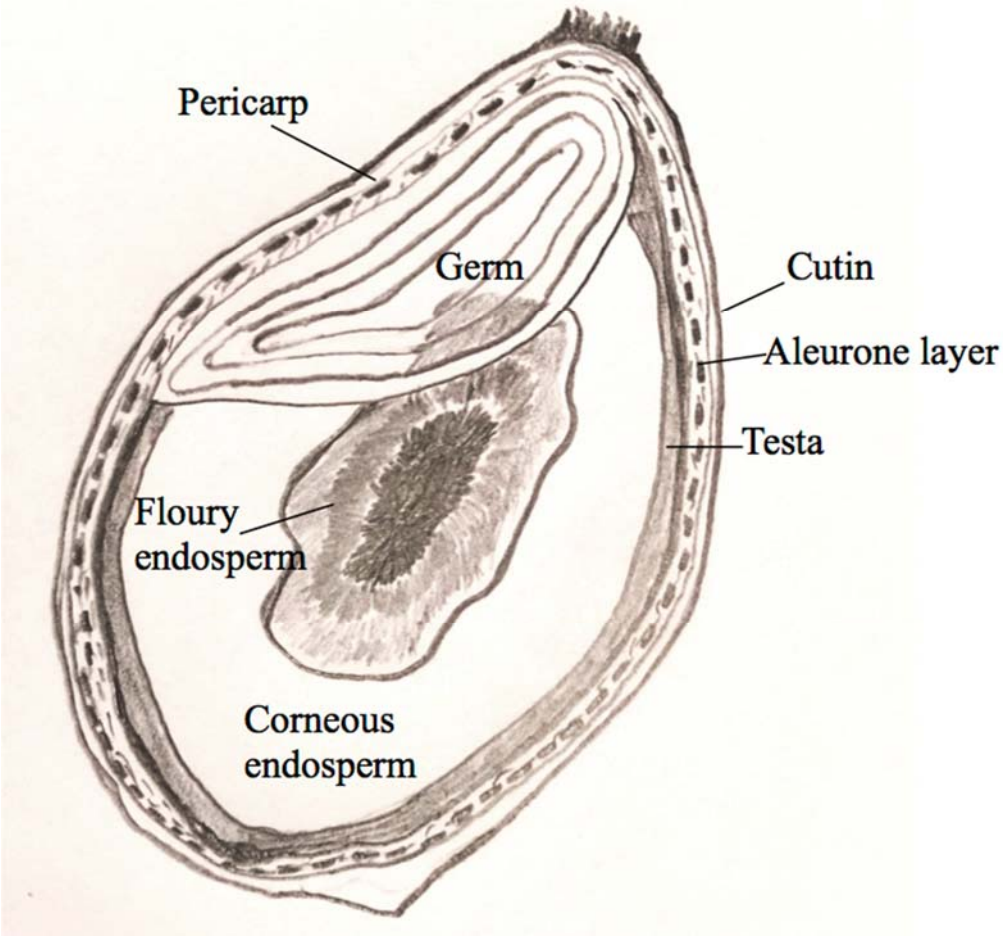

Figure 1.1 Sorghum seed morphology.

Chapter 2 - Effects of Milling Sorghum into Fractions on Yield, Nutrient Composition, and Their Performance in Extrusion of Dog Food

Abstract

The pet food market is constantly growing and worldwide is estimated to reach $95.7 billion by 2017. The US makes up over a third of this market. There is need to explore novel ingredients to support this continued market demand. Sorghum fits this purpose well. It is a non-genetically modified organism (GMO), gluten free, grows in semi-arid regions, and resists climate changes. It also may provide health benefits due to its polyphenols that are abundant in the seeds pericarp. A locally grown red sorghum was purchased for the study. It was milled into different fractions that were later incorporated into extruded dog foods. Yields of flour, mill-feed and germ averaged 69.2%, 28.5% and 0.79%, respectively. Three diets were produced from the sorghum milled fractions: a whole sorghum (WSD), sorghum flour (FLD), sorghum mill-feed (MFD). These were compared to 1:1:1 corn, rice and wheat (CON). The different starch ingredients inclusion averaged 64.5% among the treatments. Diet extrusion was organized in a completely randomized design with samples collected as repeated measures over time. Extrusion parameters were adjusted between treatments in an attempt to obtain similar expansion. The MFD required higher ($P < 0.05$) extruder water (10.17 kg/h) and extruder shaft speed (383.7 rpm), and lower feed flow rate (133 kg/h) when compared to the other diets. The bulk density off the extruder (OE) and off the dryer (OD) for MFD was 1.49 and 1.37-fold higher ($P < 0.05$) than that of FLD and WSD, respectively. The FLD had the greatest ($P < 0.05$) expansion, and was 1.92-fold more than MFD and 1.35-fold more than WSD. FLD and MFD kibbles were harder (*P*

< 0.05), and CON and WSD were similar to MFD. The diet with higher bran content (MFD) had more difficulty expanding; whereas, diets containing sorghum flour, whole sorghum and the control diet were more stable during processing. The sorghum flour, and sorghum mill-feed produced in this experiment were able to be included at high levels and produce quality kibbles for feeding to dogs.

Abbreviations: CON, control diet; WSD, whole sorghum diet; FLD, sorghum flour diet; MFD, sorghum mill-feed diet; PC, preconditioner; EX, extruder.

Introduction

The pet food market is constantly growing worldwide. In the US it comprises over one-third of the pet supplies industry and it represents more than one-third of the world's pet food market, which is forecast to reach $95.7 billion by 2017 (Taylor, 2012). This growing market is constantly changing and searching for improved nutrition and novel ingredients to satisfy consumer demand, fulfill their expectations for new, and simultaneously improve pet health.

Whole sorghum grain is not new to the pet food industry, but sorghum milled fractions are. Sub-fractions of the grain may provide benefit to animal health, food processes, and in turn produce more value. Sorghum is the fifth most important cereal crop in the world (Reddy et al., 2010). It is gluten-free, non-genetically engineered (also known as non-GMO), grows in semi-arid environments, and resists climate changes (Lemlioglu-Austin, 2014). Phenolic acids are concentrated in the testa and pericarp of all sorghums. These compounds are known antioxidants and could improve antioxidant capacity and overall health in humans and animals (Awika and Rooney, 2004). The sum of these attributes make sorghum an interesting crop to be further explored for more opportunities in the dynamic pet food market.

Most sorghum used in pet food is whole, but sorghum possesses different components within their seeds that may provide benefit in pet food if properly separated and characterized. For example, sorghum germ is high in fat and protein, while sorghum endosperm is abundant in starch, and sorghum bran is high in fiber (Serna-Saldivar and Rooney, 1995). Like corn which is milled into starch, gluten, oil and fiber fractions, and wheat is milled into starch, flour, gluten, germ oil and bran to extract greater value, so too could sorghum be deconstructed to the advantage of today's pets, with new nutrition and functional properties. The pet food industry is aggressively searching for new ingredients, so higher value fractions of sorghum might provide benefit for this market. To date very limited data are available which explore the milling of sorghum and subsequent incorporation of sorghum fractions into pet food. Therefore, the objectives of this study were to determine the effect of milling a locally grown red sorghum and processing its fractions into complete extruded kibbles for dogs.

Materials and Methods

Preliminary Milling Study

Sorghum

Before milling sorghum to be used in the study, an initial pilot-scale milling was performed in 2014 with 4,500 kg of red sorghum (2013 crop year) sourced from local producers. The sorghum was stored in totes until milling and grinding in the Hal Ross Flour Mill (HRFM, Kansas State University, Manhattan, KS, U.S.A.) were executed. Laboratory scale milling was later conducted with red sorghum purchased from AgMark LLC (Concordia, KS, U.S.A.) on March 2015, which was the same sorghum used to produce dog diets.

Milling

Initial pilot-scale milling was performed in July 2014. This step was undertaken to evaluate the capabilities of the HRFM as a first production test since its commissioning.

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 and conditioned with water to raise the moisture level around 2 percentage units. Increasing moisture content to 15–17% facilitates the separation of starchy endosperm cells from germ and hull. Also, 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 rolls 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 1st and 2nd break at approximately 20% release on 1st and 65% release on 2nd 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 go to purifiers to be cleaned. The coarser particles went to the third break which had a target release of 60-80%. The bran particles 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 the fifth break, the bran was clean of all endosperm, at which point it was removed from the milling system through the sifter and sent to 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 were sent to the sizing system where the particles were 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 were conveyed 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%) of the starch granules was mechanically damaged during milling at the reduction passages.

Quality and tailing are an intermediate step of the 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 were too dirty to be sent into the reduction passages so the product was ground on the collection passages to reclaim any remaining flour. The bran was sifted off and sent to the bran bin. Sorghum germ was removed on sifters as well after the collection passages, quality and tailing, and it was flatten down into large flakes. The total yield of bran, shorts (finer bran), red dog (overs of the last flour cloth in the mill) and some course flour were combined and called "mill-feed."

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, U.S.A.) at Shellenberger Hall (Kansas State University, Manhattan, KS, U.S.A.; Table 2.2). In this procedure, the red sorghum was cleaned and conditioned to 16% moisture content prior to lab milling. The lab milling was set-up and designed to mimic the HRFM process. The sorghum was 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 sizes through multiple grinding and sifting steps. There was no purification step in the laboratory milling.

The milling process is complex and it basically consists of a series of sizings and reductions steps until the final products are obtained. To aid in understanding this process, a diagram was made (Figure 2.1). More information about the mill settings is displayed on Table A.1 in the Appendix section.

Fraction nutrient values

The yield of fractions from the first milling were calculated dividing the weight of the fraction in question by the weight of total fractions obtained. The initial whole sorghum to the mill was not used in the yield equation because some unknown weight of sorghum was wasted to flush the system. The measured proximate analysis of sorghum fractions were moisture (AOAC 930.15), crude protein (AOAC 990.03), crude fat (AOAC 945.16), crude fiber (AOCS Ba 6a-05), and ash (AOAC 942.05; Agricultural Experiment Station Chemical Laboratories, Columbia, Missouri; Table 2.1). Fractions from the preliminary milling study were not used to produce experimental diets, but their proximate analysis were used for diet formulation.

The yields of laboratory milling were calculated by dividing the fraction in question by the total whole sorghum that was initially taken to the first roller mill (Table 2.2). Four replicates were milled and their average and standard deviations were reported.

Milling for Pet Food Study

Sorghum

A total of 2,545 kg of red sorghum were purchased from AgMark LLC (Concordia, KS, U.S.A.) in 2015 (2014 crop year) and stored in totes until milling and grinding were performed at the HRFL.

Milling

The red sorghum was milled on April 28, 2015 at the HRFM. An amount of 1,525 kg, approximately 2/3 of the total whole sorghum, was milled in order to separate flour, mill-feed,

and germ. The milling process repeated what was done in the preliminary milling study. The remainding sorghum (545.5 kg) was ground in a hammermill using a #16 standard sieve (1.191 mm) to produce whole sorghum meal for diet production of the whole sorghum dietary treatment (WSD). The whole sorghum flour was passed through a sifter after being ground in a hammermill. 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 hammermill for further grinding.

Fraction nutrient values

The red sorghum fraction yields used for diet production were calculated in the same way as the laboratory milling described above. The nutrient analyses conducted at Midwest Laboratories (Omaha, Nebraska) were moisture (AOAC 930.15), crude protein (AOAC 990.03), crude fat (AOAC 945.16), crude fiber (AOCS Ba 6a-05), acid detergent fiber (ADF; ANKOM Tech. Method), neutral detergent fiber (NDF; ANKOM Tech. Method), total dietary fiber (TDF; AOAC 991.43; mod), insoluble and soluble fibers (AOAC 991.43; mod), lignin (AOAC 973.18), total starch (AACC 76-11; mod), and ash (AOAC 942.05; Table 2.3).

Pet Food Extrusion

Diet composition

The diets were formulated to be iso-nutritional based on carbohydrate, lipid, protein, and mineral content using red sorghum fractions obtained from milling: whole sorghum diet (WSD), sorghum flour diet (FLD), sorghum mill-feed diet (MFD), and a control diet made with a combination of corn, wheat and rice in a ratio of 1:1:1 (CON; Table 2.4). Chicken fat was purchased from a regional supplier (IDF, Springfield, MO) and preserved with a commercial

antioxidant containing BHA, propyl gallate and citric acid. Red sorghum was purchased from a local Kansas mill as described above. All other ingredients were purchased from a local mill that supplies ingredients to the pet food industry (Fairview Mills L.P., Seneca, KS, U.S.A.). A single batch of the three were produced for each dietary treatment and dosed with chromic oxide (0.25%) and titanium dioxide (0.40%) as external markers in order to estimate fecal output by the dogs. The other two batches of each diet that did not contain markers were used for sensory evaluations (KSU Sensory Analysis Center; Ice Hall, Kansas State University, Manhattan, KS, U.S.A.) and palatability testing in a home setting and at a commercial kennel.

The nutrient composition of dietary treatments were determined in the Midwest Laboratories (Omaha, NE): moisture and dry matter (AOAC 930.15), organic matter and ash (AOAC 942.05), crude protein (AOAC 990.03), fat by acid hydrolysis (AOAC 954.02), crude fiber (AOCS Ba 6a-05), total starch (AACC 76-11; mod), starch gelatinized (AACC 76-11), and minerals calcium, phosphorus, potassium, magnesium, sodium, sulfur, copper, iron, manganese, and zinc (AOAC 985.01; mod).

Mixing and Grinding

The mixing, grinding and extrusion were conducted at the Bioprocessing and Industrial Value Added Program (BIVAP) facilities at Kansas State University, Manhattan, KS, U.S.A. Ingredients were weighed on a digital scale and added to a 227 kg paddle mixer in the order of the ingredients with the highest to the least inclusion. All micro-ingredients (<1% inclusion), including the markers were weighed together and added to the ration last and the ingredients were mixed for 5 minutes. All the dry ration quantity mixed was overestimated in order to compensate for any production issues that might arise, to allow the machine to become fully heated throughout, and to create replicates for analysis. In total one batch (227 kg) for each of

the diets with markers and the control diet without marker, and two batches (136 kg) for diets without markers were mixed. Following the mixing, rations were ground in a hammermill (Fitzmill, Elmhurst, IL, U.S.A.) to pass a 840 μm screen size.

Extrusion Processing

The diets that contained markers along with the mill-feed diet without marker were produced on May 13, 2015, and the remaining diets without markers were produced on May 14, 2015. Each ground ration was taken to a bin with feeder speed of 13 rpm, and conveyed to a pre-conditioner with 2 shafts (Wenger DDC Model 2; Wenger Mfg, Sabetha, KS, U.S.A.) to improve homogenization and add some moisture and heat. After preconditioning, each ration was conveyed to a single screw extruder (Model X-20; Wenger Mfg, Sabetha, KS, U.S.A.), using a typical pet food screw profile (Figure 2.2) that 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. At the end of the extruder barrel, the die hole diameter was 7 mm and kibbles were cut with 6 knives blades.

Bulk density was measured using a 1L cup and a scale with 0.01g sensitivity. The cup was filled with kibbles and the top layer of the product was gently leveled by hand. The full cup was weighed and bulk density recorded. There were 2 bulk densities measured: bulk density out the extruder (OE) and out of the dryer (OD). Product flow rate was measured by filling a 4L container with all kibbles exiting the extruder during 1 min. Weight was recorded and the units were converted to $kg \times s^{-1}$. All measurements and extruder control information were recorded every 20 minutes during extrusion. Specific mechanical energy (SME) was calculated using the equation 2.1 (Yoo et al., 2011):

$$SME\ (kJ/kg) = \frac{(\tau - \tau_0)/100 \times (N/N_r) \times P_r}{m} \quad (2.1)$$

Where τ is the % torque or motor load; τ_0 is the no load torque (32% for the X20 extruder); N is the screw speed; N_r is the rated screw speed (508 rpm); P_r is the rated motor power (50 kW); and m is the mass flow rate or feed rate (kg/s).

Post-extrusion, kibbles were pneumatically conveyed to a dual pass dryer-single pass cooler (Model 480; Wenger Mfg, Sabetha, KS, U.S.A.). The dryer was set at 123.8°C and kibbles were conveyed for 8 minutes per pass (16 min total) and 5 minutes through the cooler. The final moisture goal for the diets was less than 10%. After drying, all the food was conveyed to a coating tunnel where the chicken fat was applied at a rate corresponding to each formulation. All coated kibbles were collected into a container before filling 9 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 calculations 2.2 and 2.3.

$$\text{Piece volume } (cm^3) = \pi \times (\text{piece diameter in cm})^2 \times (\text{piece length in cm})/4 \quad (2.2)$$

$$\text{Piece density } (g/cm^3) = (\text{piece mas})/(\text{piece volume}) \quad (2.3)$$

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 5 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%. 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% was the calculated area under the curve (N×mm) of each compression signature.

Statistical analysis

Extrusion data were analyzed as a completely randomized design (CRD) with repeated measures over time. Four diets were treated as fixed effects under each of the two markers, which were treated as fixed random blocking effects. There were 2 levels in the blocking factor for each diet: 1) diet with green marker Cr_2O_3 and 2) diet without marker. Within each of the marker by diet combination, responses were measured at 3-5 time points at a 20-min interval dependent on type of response and sample availability. At each time point, five subsamples of randomly selected kibbles were measured and weighed to calculate multiple responses, such as piece volume, piece density and SEI, and then averaged to represent each time point. The same procedure was done for texture analysis. Heterogeneous variances were considered among diets and time points using variance component (VC) type variance-covariance structure followed model selection using Bayesian Information Criteria (BIC); wherein, a distinct variance component was assigned to each diet effect. Dietary treatment least square means were separated using the Tukey Type I error correction method when overall tests of diet effects were significant at an $\alpha = 0.05$ significance level. Analysis was conducted using GLIMMIX procedure in Statistical Analysis System (SAS version 9.4; SAS Institute, Inc., Cary, NC, U.S.A.). Feed screw speed was the only extruder variable where it was not possible to use repeated measures because there was no time-to-time variability for this particular response. Hence for feed screw speed the time points were averaged and analyzed as a CRD using GLIMMIX.

Results

Preliminary Milling Study

For the preliminary milling the yields of flour, mill-feed and germ were 70.02%, 29.73% and 0.33%, respectively (Table 2.1). After milling, moisture of whole sorghum was numerically the highest (14.0%), followed by flour, germ and mill-feed (12.8,%, 10.6% and 10.7%, respectively). Crude protein was numerically higher in germ (16.26%) and mill-feed (14.87%), and lower in flour (9.23%) and whole sorghum (10.90%). Crude fat was lower in whole sorghum and flour (1.69% and 0.03%), and higher in mill-feed and germ (5.19% and 6.18%). Crude fiber was higher in mill-feed (5.11%) and decreased from 2.78% to 2.36% to 0.88% in whole sorghum, germ and flour, respectively. Finally, ash content ranged from 0.94% to 2.79%, being the most concentrated in the mill-feed and germ and lowest in flour.

The four sorghum samples milled in the laboratory had a lower average flour yield (49.97%) compared to both pilot scale millings, whereas average mill-feed yield was 38.50%, average germ yield was 2.93% and there was an 8.59% loss (Table 2.2).

Milling for Pet Food Study

For diet production sorghum fractions were produced at the HRFM and the milling yields were similar to those of the first large scale milling (Table 2.3). Finished flour yield was 1,041kg, which represented 68.3% of the total, while mill-feed had a yield of 414kg (27.2%). Germ fraction was estimated to be 1.25% of the total and was too small to be considered for diet production, so it was discarded. The initial moisture was lower than the whole sorghum moisture of the first milling (12.22%; Table 2.3 vs 14.00%; Table 2.1) and it was better distributed between fractions; wherein, flour and mill-feed had 11.59% and 10.87% of moisture,

respectively. The crude protein content was, as expected, higher for the mill-feed fraction (13.9%) and lower for flour (10.2%). Crude fat differed significantly from the first milling: whole sorghum, sorghum flour and mill-feed crude fat ranged between 4.96% and 6.59%, which is high for the first two fractions. Acid detergent (ADF) contents were 4.0%, 1.7% and 8.2% and neutral detergent (NDF) contents were 6.70%, 1.50% and 16.80% 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% was insoluble fiber. Total dietary fiber from sorghum flour was the lowest numerically (3.20%), composed of 2.50% soluble fiber and only 0.70% insoluble fiber. Conversely, sorghum mill-feed had, as expected, the highest numerical quantity of TDF (20%), which consisted of 1.6% soluble fiber and 18.3% insoluble fiber. Moreover, sorghum mill-feed was the only fraction that had a detectable fraction of lignin of 2.90%. Total starch for mill-feed was numerically lower than that of whole sorghum and sorghum flour (43.8% vs 61.5% and 67.0%, respectively). Lastly, ash content was similar to the first milling (Table 2.1); wherein, mill-feed had slightly higher ash percentage.

Pet Food Extrusion

Diet Development

The starch ingredients of all diets averaged 64.5% (table 2.4). Chicken by-product meal and chicken fat were adjusted according to the nutritional composition of each starch treatment, and averaged 20.5% and 4.92% respectively. Beet pulp and corn gluten meal were incorporated in the same proportion in each diet and all the minerals and vitamins were added in a similar proportion.

The moisture, dry matter and organic matter did not differ among treatments and averaged 5.92%, 94.0% and 93.2%, respectively (Table 2.5). Crude protein content was slightly higher ($P < 0.05$) for CON and MFD than for the FLD treatment, which was similar to WSD. Crude fat concentration was also different between CON and MFD, with WSD and FLD intermediate to each. The MFD had a crude fiber content of 2.17%, which was higher than that of CON and FLD, and numerically greater than WSD. Moreover, the MFD had the lowest ($P < 0.05$) starch quantity (35.3%), and CON, WSD and FLD starch content were similar and averaged 47.5%. From the total starch, FLD and MFD had the highest (< 0.05) gelatinization (average 94.9%); whereas, starch gelatinized from CON and WSD was lower and averaged 85.6%. The vitamins and minerals were relatively consistent among diets.

Extrusion Processing

Product flow rate for CON, WSD and FLD did not differ (average 151.5 kg/h) and was lower ($P < 0.05$) during the MFD production (131.9 kg/h). Density of kibbles exiting the extruder (OE) was heavier ($P < 0.05$) for MFD, intermediate for CON and WSD and lighest for FLD. Likewise, kibble density exiting the dryer (OD) was also heavier ($P < 0.05$) for MFD, with CON and WSD intermediate, FLD similar to CON and WSD similar to MFD.

The feed screw speed and preconditioner steam were not different among treatments (averages 13.2 rpm and 17.5 kg/h, respectively). The preconditioner water was greatest during production of CON, WSD and FLD (average 15.7 kg/h), and lowest during production of MFD (13.3 kg/h). The temperature of material in the preconditioner was greater ($P < 0.05$) for WSD than MFD, with CON and FLD similar and intermediate to the extremes. Extruder shaft speed was faster ($P < 0.05$) during MFD extrusion (383.7 rpm) than the other treatments, which averaged 320.6 rpm. Extruder steam was higher during extrusion of CON and WSD (average

19.4 kg/h) than when processing FLD and MFD (average 4.26 kg/h). Motor load was similar among treatments and averaged 45.1%. Addition of water in the extruder was greater for MFD production (10.17 kg/h) than the other treatments (average 7.69 kg/h). Knife speed and SME were not different among diets and averaged 909.6 rpm and 103.0 kJ/kg, respectively.

Measurements related to kibbles after drying (before coating) are reported in Table 2.8. Piece diameter exiting the dryer was smallest ($P < 0.05$) for the MFD and larger for WSD followed by CON, with the greatest radial expansion ($P < 0.05$) for the FLD dietary treatment. Piece length for CON, WSD and FLD were longer ($P < 0.05$) than FLD, which was similar to CON. The MFD kibble mass was lowest among the treatments. Piece volume was also lowest ($P < 0.05$) for MFD treatment compared to the others. Piece density was, as expected, heavier ($P < 0.05$) for MFD (0.467 g/cm^3), intermediate for WSD and CON (average 0.377 g/cm^3) and lowest for FLD treatment (0.334 g/cm^3). 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 ($P < 0.05$), with FLD being more expanded than CON and WSD being more expanded than MFD. Energy required to break the kibbles by 50% was similar among diets and averaged 144.3 N*mm, whereas piece hardness was highest ($P < 0.05$) 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.

Discussion

Sorghum Milling

The Hal Ross Flour Mill had never milled sorghum before this study, so it was important to have an understanding of required facility settings and yields for this grain. The yield of flour

at 70% 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; however, capturing a pure bran stream (pericarp, cutin, alurone layer and testa) was not feasible for yield and mill capabilities. So the mill-feed served the purpose for a bran-rich fraction knowing that it contained some endosperm as well. Finally, there was every intention to collect sufficient amounts of germ to incorporate into research diets for one of the experimental treatments. However, the yield at less than 1% was not adequate to allow sufficient material to produce foods from extrusion processing. Thus, this treatment was abandoned and the material was included in the mill-feed.

The whole sorghum moisture as received was expected to exceed 12% as is typical for stored grain (Table 2.1) and this agrees with the moisture content Vargas-Solorzamo et al. (2014) found in white, red and brown pericarp sorghums. The moisture content was numerically lowest in the mill-feed fractions of both millings (Tables 2.1 and 2.3) because all the components of mill-feed (shorts, red dog, bran and some coarse flour) took longer to exit the system. Thus, more water was lost through the vents. Likewise, the crude protein matched what was expected for the whole sorghum and sorghum flour. The crude protein was concentrated in the fibrous mill-feed fraction and germ. The aleurone layer in the pericarp and the peripheral endosperm tissue, which would be part of the sorghum mill-feed, contain cells with a large amount of protein (Serna-Saldivar and Rooney, 1995). Crude fat values were also consistent with those expected in the whole sorghum and sorghum fractions, with particular note regarding the decline to near 0 (0.3%) in the flour. The balance of the crude fat was distributed among the mill-feed and germ; each with the highest quantities. Moraes et al. (2015) also found that sorghum bran was the fraction with highest crude protein, lipids and fiber content, and the flour least among these

nutrients. Germ is the embryonic tissue and contains large reserves of protein and fat (Serna-Saldivar and Rooney, 1995), thus it was anticipated to contain higher proportions of crude fat and protein (Table 2.1). There is need to revisit the mill settings in order to better capture a greater proportion of this fatty germ and perhaps more value. Finally, the ash content shifted away from the flour and into the mill-feed and germ fractions, but the amount was not significant as it relates to companion animal nutrition. Additional flour milling evaluations in the lab were undertaken to work through some of the milling issues described above (Table 2.2). In the lab sorghum was milled into the same 3 fractions and "lost material" was also quantified. The average yield of flour across four replications was just short of 50%. This is much lower than noted for the initial HRFM test. Moraes et al. (2015) decorticated sorghum in a laboratory impact mill using a rice-polisher and reported flour and bran yields of 54.4 and 27.8%, respectively. These results were similar to what was obtained in the laboratory milling of our study. The mill-feed fraction from laboratory milling was relatively close (~35%) to that observed in the prior HRFM test; and the germ yield was much improved. The lab data differed substantially from actual production results, most probably due to milling efficiency. A large commercial mill is capable of grinding harder on the reduction rolls and also has pin mills and flake detachers which greatly aid in reducing endosperm particle size and flakes right after the reduction roll and before the sifter. Also, in the lab milling there were no purification steps, which could also impact the results.

With the information gathered from the initial milling and lab milling, a final sorghum milling session 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 with nearly 70% flour, nearly 30% mill-feed and germ in quantities too small to use for experimental diet production. The sorghum fraction yields produced in the 2015 crop samples (Table 2.3) may have been underestimated for germ, mill-feed or both as approximately 3% of the total is missing. Quite possibly, some germ, course flour, or another small sorghum fraction that was incorporated into the mill-feed did not get counted.

The whole sorghum nutritional composition somewhat agrees with red sorghum nutritional values described by Jaworski et al. (2015); wherein, starch content was lower in our work (61.5%; Table 2.3 vs 69.0%), crude protein was 1.8% higher, and total dietary fiber (TDF) was similar (8.80%, Table 2.3 vs 8.30%). However, the insoluble fraction from our work corresponded to 70% of the TDF, while Jarwoski et al. (2015) reported over 93% of insoluble fiber in red sorghum. Nevertheless, all the nutrients analyzed for the red sorghum from the present study lie within the nutritional composition ranges of several sorghum genotypes reported by Queiroz et al. (2015). Frederick (2009) milled a white sorghum genotype at 60%, 80% and 100% flour extractions and for all three extractions the author reported less fat, less protein and more starch compared to our red sorghum. According to Bach-Knudsen and Munck (1985), sorghum fiber fraction is primarily insoluble (6.5 to 7.9% insoluble and 1.1 to 1.23% soluble β-glucans), and agrees with our work. The mill-feed fraction was a mixture of some endosperm, bran, shorts, and red-dog. Around 86% of the insoluble fiber fraction is present in the pericarp (Waniska and Rooney, 2000). Besides the high percentage of insoluble fiber in sorghum mill-feed fortunately enough starch was retained (43.8%) to make extrusion processing possible.

Pet Food Extrusion

The dietary treatments were 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 in pet food (Table 2.4). 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 formulas were balanced to be 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 supplied 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 (AAFCO, 2016). In addition to the nutritional constituents, diets fed to dogs in the digestion study were produced with external markers (chromium sesquioxide and titanium dioxide) incorporated to allow their use in estimating fecal output for the computation of apparent total tract digestibility for 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 met the expected levels predicted from the initial formulation (Table 2.5). Each of the experimental treatments had a moisture and (or) dry matter level that was consistent with a typical dry extruded pet food (below 10%). The crude protein was similar among the CON, WSD, and FLD diets, but was somewhat higher for MFD. The fiber content was also higher in the MFD. This should be expected based on the nutrient composition of sorghum bran reported by Moraes et al. (2015). The amount of fat on the product was somewhat controlled by the

topical addition of chicken fat, but despite this the control diet had a higher level of fat than each of the sorghum containing foods. It must be taken into consideration that kibbles were coated in a tumble drum tunnel and fat was dripped onto kibbles, which may have resulted in inconsistent distribution for all pieces. The crude fiber composition of the diets was expected to be higher in the MFD; but, relative to commercial pet foods this would not be considered 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) at the extrusion laboratory in BIVAP (Tables 2.6-2.8). In extrusion, or any food processing, some of the finished product characteristics are related to the processing parameters. For example, regardless of whether exiting the extruder or the dryer, the MFD kibbles were denser and less expanded than those from the other treatments. The higher density, or lack of expansion, may be consistent with the lower starch level and higher insoluble fiber of sorghum mill-feed composition. The MFD also had a lower flow rate compared to the other treatments. Turner (2004) also found that extrudates with addition of sorghum bran had decreased flow rate and heavier density as outcomes.

The screw speed and preconditioner steam were consistent among treatments (Table 2.7). The preconditioner water was adjusted during production in an attempt to achieve consistent product physical parameters. Preconditioning prior to extrusion will plasticize the raw materials with heat and moisture due to added steam and water, which significantly contribute to energy input and retention time (Strahm, 2000). This step reduces extruder mechanical energy input and component wear, and also the increased retention time in the preconditioner allows the raw material particles to be mixed and penetrated with water. An efficient homogenization and water

absorption are important steps for pet foods; especially, when extruding more complex formulations. The MFD was a more complex formulation compared to the other diets due to its higher bran and lower starch contents. Despite this attribute MFD received less preconditioner water and had the same retention time as the other treatments (same feed screw speed). However, this was not a problem since the MFD had a high percentage of gelatinized starch, indicating that it cooked well. All diets had a satisfactory starch gelatinization, which is directly related to starch digestibility (Asp and Björck, 1989; Camire, 2000).

The extruder shaft speed was slightly faster during the mill-feed dietary treatment. An increased shaft speed will add more mechanical energy in the process through shear, which helps cook the food. But, there can be a drawback when operating at high shaft speeds. Specifically, when the extruder is not running at full capacity with a high shaft speed there may be surging (Riaz, 2000b) and this can impact product uniformity and product density. Similar to what was observed in the MFD treatment that had the lowest motor load. Extruder steam is important to add thermal energy to the process. Extruder steam was lower for the MFD and FLD, and higher for CON and WSD treatments.

Specific mechanical energy (SME) is a good quantitative parameter to estimate the amount of mechanical energy delivered to the extrudate which will directly impact macromolecular transformations, interactions between molecules, and rheological properties of the melt (Moraru and Kokini, 2003). In the present study, SME was numerically lower during production of MFD (Table 2.7). Turner (2004) also observed that SME decreased with the addition of sorghum bran in snacks. The lower SME in MFD was likely due to the lower flow rate (Table 2.6) and the numerically lower motor load (values used to calculate SME). According to Vargas-Solórzamo et al. (2014) SME is related to the viscosity of molten mass. These authors

claimed that linear polysaccharides of high molecular mass, such as fiber and amylose, increase the viscosity of the molten mass, which would affect the resistance performed on screws and increase SME. Apparently, the fiber content of MFD was not enough to increase the SME. Vargas-Solórzamo et al. (2014) also reported that lower SME was related to coarse sorghum (≤ 1 mm). Although sorghum fraction particles in the present experiment were intended to be the same size, sorghum mill feed may have been coarser. A particle size study would be suitable to further investigate if this claim was valid.

When dealing with extrusion of complete pet foods there are many factors involved, due in large part to the complexity of the formulations. In this study the effects of other non-starch ingredients on SME, expansion, density and other parameters related to the process should not have had an impact due to their consistency between treatments. Other factors that were not measured but which could impact the process were the pre-conditioner and extruder hardware, extruder operator and operating conditions.

Besides collecting and measuring parameters during extrusion it is also important to evaluate kibble density and expansion, since these attributes can influence kibble surface and cell size. These factors are directly related to fat absorption in the coating stage. Coating can add some nutrients to the kibbles such as vitamins, minerals and amino acids, and is important in increasing palatability and textural attributes of the kibbles (Corrigan and Sunvold, 2010). The FLD had higher kibble expansion than CON, followed by WSD (Table 2.8). The MFD on the other hand had the lowest expansion and heaviest piece density, suggesting that its lower starch content may have played a role on the process. An increased starch content, extrusion temperature, and gelatinization cause greater expansion (Mercier and Feillet, 1975; Geetha et al.,

2014). To this end the FLD had highest starch content, highest gelatinization, and this led to a greater expansion.

As mentioned before, extruder shaft speed and extruder water during MFD production were the highest among treatments, which could have led to a decreased SEI. Robin et al. (2012) found that SEI of extruded whole wheat flour decreased when increasing screw speed, extruder water, and in-barrel temperature, while all other process parameters were held constant. The fiber level is also important in extrusion cooking. Grenus et al. (1993) found that radial and axial expansion of rice flour extrudates increased up to a 10% bran level and then decreased at higher levels. The same pattern was observed in the present study; wherein, whole sorghum diet had a satisfactory expansion, and MFD, with increased level of bran, did not expand as much. According to Moraru and Kokini (2003), fiber molecules above a critical concentration disrupt the continuous structure of the melt and also trap some of the water, reducing expansion.

Kibble uniformity and a proper expansion are important because, besides improving fat absorption and palatability, it also affects appearance, hardness and product uniformity. These are attributes related to client and pet acceptance. There are instrumental testing devices that attempt to compare and quantify human sensory perception. They rely on transducers to convert material and physical measurements into visual or electrical outputs. Measures by transducers may represent physical characteristics of the product in terms of absolute units, and this is valuable because human perception is evaluated by psychophysical phenomena that are difficult to measure (Rosenthal, 1999).

Hardness in this experiment was considered the peak force of each kibble compression, which was defined in the same manner as Geetha et al. (2014). Energy for compression in the present study was the energy required to compress the kibbles to 50% strain. Brnćič et al. (2006)

investigated the effects of processing parameters on the mechanical hardness of extrudates using a twin-screw extruder and these authors found that feed moisture had a positive effect on extrudate hardness, while temperature and screw speed had negative effects. The FLD was harder than CON and WSD and this may be in part explained by the lower thermal energy input due to its lower extruder steam addition. The MFD also had lower steam addition in the extruder, which may have impacted its hardness, but since it had high variance it was grouped as not different from all diets. The higher quantity of extruder water required for MFD production may partially explain its hardness and lack of expansion. Water acts as a plasticizer reducing the viscosity and mechanical energy dissipation, thus the product becomes dense and bubble growth is compressed (Ding et al., 2006). It was expected that the FLD would have lower hardness because it expanded the most, but this was not the case. Koppel et al. (2014) reported a similar finding using a sensory panel; wherein, hardness was defined as the force required to bite completely through the sample with molar teeth, and baked products that were denser actually had lower hardness. Hardness is related to the diet recipe, and more prominently to processing conditions and the development of kibble cell wall structure. What remains to be investigated is if a harder or a softer product of same moisture is preferred by dogs. There is indication that fiber addition to extruded dry kibbles increases hardness, bitterness, and may negatively affect palatability for dogs (Koppel et al., 2015).

In conclusion, extrusion is a complex process that is influenced by numerous factors. It is important to measure and analyze parameters during and after extrusion, as tools to understanding the process and provide the operator controls over the changing variables in order to obtain the desired product parameters. Due to the higher fat and protein composition, sorghum germ would be an interesting fraction to be studied in extruded pet food in the future.

Summary

Sorghum milling resulted in higher flour yield in the pilot scale than in the laboratory, and sorghum fractions obtained at the Hal Ross Flour Mill (HRFM) had nutrient compositions that were expected. The first and second millings conducted at the HRFM had very similar yields, and flour separation was efficient (ca. 70%). However, there was an underestimation of both mill feed and germ, with 3% of the total lost or uncounted. Since the goal of milling is to obtain a flour fraction from the grain, there is commonly no concern regarding the germ or mill feed in sorghum. But if the germ yield was improved it could be used as another fraction in pet food production.

Diets for the study were formulated to have similar nutrient composition and to contain the same ingredients except the starch sources. The extrusion was performed on a single-screw extruder (Model X-20; Wenger Mfg.) and the goal was to obtain similar kibbles expansion among diets while trying to keep processing conditions equal or similar. All diets expanded well with the exception of MFD. The lower expansion may be attributed to the high fiber content of the mill-feed fraction, and in part due to extruder operating conditions and extruder hardware. Extrusion parameters were collected and some were used to calculate SME, which was numerically lower for MFD. Sorghum flour diet (FLD) had the largest expansion. Kibble expansion is important in pet food because it provides textural attributes and coating uniformity, which can directly impact client and dog acceptance, and product shelf-life. Understanding extrusion performance and knowing how to control the process is essential to obtain the desirable product. There is need to better characterize extrusion parameters to produce uniform diets that contain sorghum mill-feed, as well as to study the effect different sorghum mill-feed level of inclusion would have on kibble expansion and other attributes.

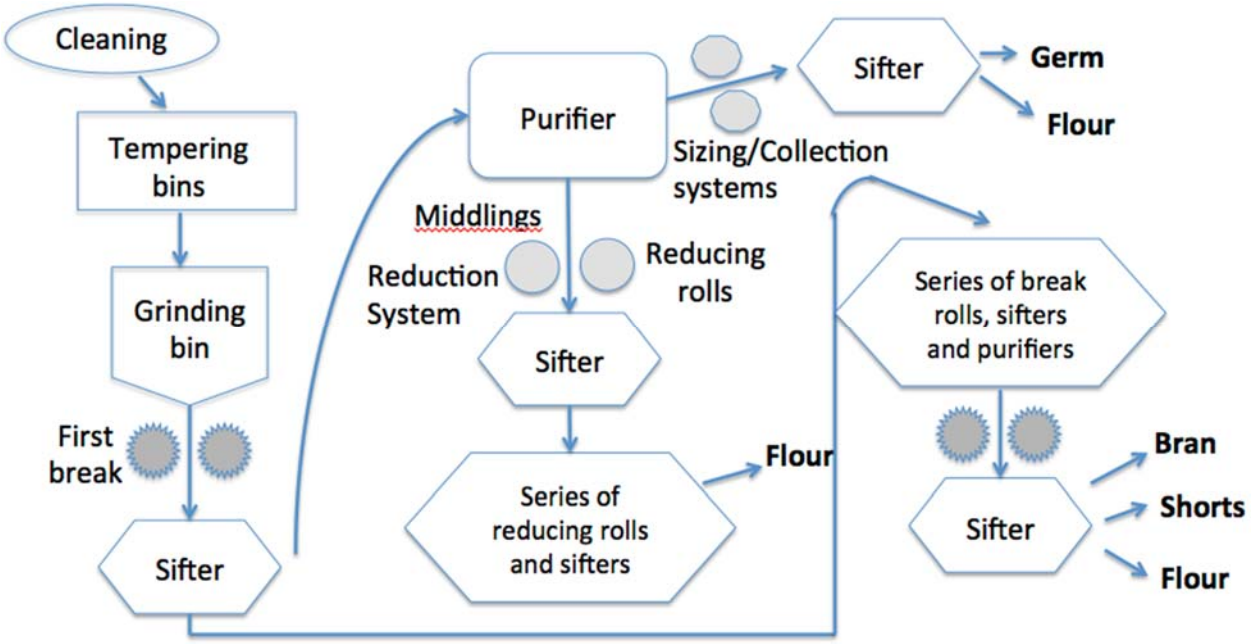

Figure 2.1 Simplified milling diagram.

Table 2.1 Yield and proximate 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).

Item	Whole sorghum	Flour	Mill-feed	Germ
Yield, kg	4,050	2,836	1,214	13.2
HRFM Yield, %	100	70.02	29.73	0.33
Moisture, %	14.0	12.8	10.6	10.7
Crude Protein, %	10.90	9.23	14.87	16.26
Crude Fat, %	1.69	0.03	5.19	6.18
Crude Fiber, %	2.78	0.88	5.11	2.36
Ash, %	1.44	0.94	2.73	2.79

Table 2.2 Yields of sorghum fractions from laboratory milling evaluations on 2014 crop-year sorghum, used to produce diets.

Item	Flour	Mill-feed	Germ	Lost
Yield, %	49.97 ± 3.487	38.50 ± 8.109	2.93 ± 0.955	8.59 ± 5.838

Table 2.3 Yield and proximate analysis on as-is basis of red sorghum used to incorporate into the dietary treatments (2014 crop-year sorghum milled on April/2015).

Item	Sorghum to the mill	Flour	Mill-feed	Germ
Yield, kg	1,525	1,041	414	-
Yield, %	100	68.3	27.2	1.25
Moisture, %	12.22	11.59	10.87	-
Crude Protein, %	10.8	10.2	13.9	-
Crude Fat, %	6.59	5.12	4.96	-
Crude Fiber, %	1.70	n.d.	5.05	-
ADF, %	3.80	1.40	7.70	-
NDF, %	6.70	1.50	16.80	-
TDF, %	8.80	3.20	20.0	-
Soluble Fiber, %	2.60	2.50	1.60	-
Insoluble Fiber, %	6.20	0.70	18.30	-
Lignin, %	n.d.	n.d.	2.90	-
Total Starch, %	61.5	67.0	43.8	-
Ash, %	1.31	1.53	2.20	-

Table 2.4 Experimental diets produced to evaluate the effects of sorghum fractions on extrusion: Control (CON), whole sorghum (WSD), sorghum flour (FLD) and sorghum mill-feed (MFD).

Ingredients, %	CON	WSD	FLD	MFD
Brewers rice	21.2	-	-	-
Corn	21.2	-	-	-
Wheat	21.2	-	-	-
Whole sorghum	-	64.7	-	-
Sorghum flour	-	-	62.3	-
Sorghum mill-feed	-	-	-	67.6
Chicken by-product meal	20.9	20.0	21.0	20.0
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 premix[a]	0.15	0.15	0.15	0.15
Trace mineral premix[b]	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

[a]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.

[b]Trace mineral premix: calcium carbonate, zinc sulfate, ferrous sulfate, copper sulfate, manganous oxide, sodium selenite, and calcium iodate.

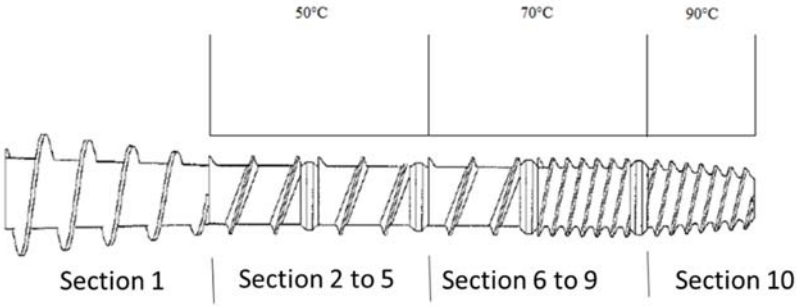

Figure 2.2 Extruder screw profile used to extrude the experimental diets control (CON), whole sorghum (WSD), flour (FLD) and mill-feed (MFD).

Table 2.5 Nutrient analysis of final experimental diets (as is) Control (CON), whole sorghum (WSD), sorghum flour (FLD) and sorghum mill-feed (MFD).

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), %	21.5[a]	21.4[ab]	21.2[b]	23.8[a]	0.43	0.0355
Fat (acid hydrolysis), %	12.10[a]	10.70[ab]	10.25[ab]	9.48[b]	0.392	0.0373
Fiber (crude), %	0.675[b]	1.330[ab]	0.725[b]	2.710[a]	0.3300	0.0346
Total starch, %	46.9[a]	45.6[a]	50.0[a]	35.3[b]	1.71	0.0140
Starch gelatinized, %	85.2[b]	86.1[b]	96.3[a]	93.5[a]	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[b]	0.135[ab]	0.100[b]	0.165[a]	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
Iron, ppm	179.0	180.5	161.5	187.0	7.06	0.2102
Manganese, ppm	27.8[ab]	25.2[ab]	19.6[b]	34.0[a]	2.24	0.0443
Zinc, ppm	143.5	159.5	136.5	165.5	15.55	0.5737

[ab] Means within a row that lack a common superscript differ ($P \leq 0.05$).

Table 2.6 Mean ± 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).

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

*OE= Kibbles collected out of the extruder; **OD= Kibbles collected out of the dryer.
^{abc}Means within a row that lack a common superscript differ ($P \leq 0.05$).

Table 2.7 Mean ± 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).

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

*FS= feed screw; **PC = preconditioner; ◆EX = extruder; •SME= specific mechanical energy.
[ab] Means within a row that lack a common superscript differ ($P \leq 0.05$).

Table 2.8 Mean ± 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).

Item	CON	WSD	FLD	MFD	P =
Piece diameter, mm	$14.2^{b} \pm 0.08$	$13.2^{c} \pm 0.10$	$15.3^{a} \pm 0.07$	$11.0^{d} \pm 0.45$	0.0047
Piece length, mm	$6.21^{ab} \pm 0.213$	$6.94^{a} \pm 0.113$	$5.81^{b} \pm 0.057$	$6.57^{a} \pm 0.052$	0.0015
Piece mass, g/kibble	$0.371^{a} \pm 0.0110$	$0.357^{a} \pm 0.0103$	$0.358^{a} \pm 0.0036$	$0.288^{b} \pm 0.0020$	0.0002
Piece volume, cm^3	$0.981^{a} \pm 0.0452$	$0.950^{a} \pm 0.0300$	$1.074^{a} \pm 0.0093$	$0.634^{b} \pm 0.0518$	0.0038
Piece density, g/cm^3	$0.379^{b} \pm 0.0064$	$0.376^{b} \pm 0.0023$	$0.334^{c} \pm 0.0012$	$0.467^{a} \pm 0.0400$	0.0003
SEI*	$4.10^{b} \pm 0.048$	$3.56^{c} \pm 0.056$	$4.81^{a} \pm 0.043$	$2.50^{d} \pm 0.200$	0.0002
Hardness, N	$90.6^{b} \pm 4.38$	$76.4^{bc} \pm 3.85$	$120.5^{a} \pm 5.55$	$121.9^{abc} \pm 19.17$	0.0120
Energy for compression, N×mm	146 ± 10.5	120 ± 8.9	160 ± 4.7	150.5 ± 14.3	0.0742

*SEI = Sectional expansion index.
^{abc} Means within a row that lack a common superscript differ ($P \leq 0.05$).

Chapter 3 - Apparent total tract digestibility and antioxidant capacity of dogs fed diets containing sorghum fractions.

Abstract

Sorghum is an abundant starch source that has many potential health benefits. Some pet food companies have adopted whole sorghum in their formulations, however sorghum flour and (or) its polyphenol rich seed coat might provide added benefit to companion animal diets. The objective of this experiment was to evaluate diets utilizing sorghum flour (FLD), and sorghum mill feed (MFD) relative to whole sorghum (WSD), and conventional grains (rice, corn and wheat; CON) in a typical dog diet. Adult (1-3 yr) Beagle dogs (n=12; 10.6 kg ± 1.4) were randomly assigned to individual pens with *ad libitum* access to water. Dogs were fed twice daily and adapted to diet (9 d), and then total feces were collected for 5 d over 4 periods in a replicated Latin square design. Fecal output was estimated and compared by 4 different methods: using Cr_2O_3 and TiO_2 as external markers, acid insoluble ash (AIA) as an internal marker and by total fecal collection (TFC). Number of defecations were quantified and feces were scored on a subjective 5-point scale. Lastly, the antioxidant activities of the tested diets in dog plasma were measured using an oxygen radical absorbance capacity (ORAC) kit. Means were separated with multivariate analysis of variance (MANOVA) using the GLIMMIX procedure of the statistical analysis software (SAS 9.1). The sorghum fractions did not affect dog acceptance (average feed intake was 187 g/d), but dogs fed the MFD had a larger ($P < 0.05$) amount of feces excreted, more defecations per day, and lower ($P < 0.05$) overall nutrient digestibility compared to the other treatments. The FLD had the highest ($P < 0.05$) values of DM, OM, Energy, and CP

digestibility, suggesting a possible application in easy-to-digest pet foods. The external markers, Cr_2O_3 and TiO_2, tended to overestimate apparent digestibility when compared to the TFC; however, external markers had a higher correlation to TFC than the intrinsic marker AIA. Interestingly, TiO_2 had a high correlation ($P < 0.05$; average R=0.93) to Cr_2O_3 (the official fecal marker for dogs and cats) and also correlated well to TFC and AIA. The study suggests that TiO_2 may be a more appropriate marker to estimate fecal output in dogs than Cr_2O_3 and the use of AIA technique needs to be further explored. The plasma ORAC value in dogs fed the MFD was 2-fold higher ($P < 0.05$) than each of the other treatments. It was expected dogs fed WSD would have higher plasma ORAC values, but they did not differ from CON and FLD. In conclusion, sorghum fractions have potential application in pet food. It is important to determine the right proportion of sorghum bran to include that will promote health, but not decrease digestibility.

Key words dog; sorghum; bran; digestibility; ORAC; AIA, titanium dioxide

Introduction

The global pet food market has had strong growth for years. It was estimated to be $65.8 billion in 2010 and is now forecast to reach $95.7 billion by 2017 (Taylor, 2012). The US makes up over one third of this dynamic multibillion dollar market with innovation fueling its constant growth. As pet ownership shifts to "pet parenthood" there is a tendency to feed pets the "best diet" to promote and improve their health. High volume industrial pet food production requires sustainable, yet novel ingredient sources be developed to support this demand.

Sorghum grain has some unique attributes that make it an interesting ingredient to be explored and expanded in the pet food industry. Sorghum is gluten-free, non-genetically engineered, grows in semi-arid environments, and resists plague and climate change (Lemlioglu-Austin, 2014). There are a few studies demonstrating that whole sorghum incorporated in pet

food led to a lower glycemic index (GI) in dogs and cats (Carciofi et al., 2004; Sunvold and Bouchard, 1998; Bouchard and Sunvold, 2000). Additionally, sorghum bran (obtained from sorghum pericarp) was found to have the lowest glycemic index (Moraes et al., 2015). It also contains phenolic compounds in the pericarp which may improve physiological antioxidant capacity (Awika and Rooney, 2004).

Despite the benefits of sorghum bran related to its high level of phenolics and potential benefit from fiber, there is an off-setting challenge due to condensed tannins (also known as proanthocyanidins) within the pericarp of some sorghums which can precipitate proteins, inhibit digestive enzymes to a certain degree, and chelate some of the trace minerals (Scalbert et al., 2000). However, the effects of tannins, or procyanidins, in the body are still not fully elucidated. Scalbert et al. (2000) suggested that "the non-specific binding of procyanidins to proteins is probably of limited nutritional significance as far as they are consumed in limited amounts." The effects of tannin-rich foods on dog digestibility and overall health have not yet been explored. Since pet food is a complex formulation commonly containing excess minerals and protein, it is expected that the benefit from condensed tannins would be more beneficial than detrimental.

Conversely, as bran is removed a starch rich flour is produced, which may yield an improved functional starch for processing and (or) increase diet digestibility for specialty foods. Each element if isolated and concentrated could potentially add volume and value to the use of sorghum based products in pet foods. Therefore, the objectives of the present study were to determine the effect of different sorghum fractions on diet acceptability, utilization, and antioxidant capacity in dogs. As a sub-objective of the study, the methods used to estimate fecal output and to calculate apparent total tract digestibility in dogs were evaluated.

Materials and Methods

Dog feeding study

Twelve intact Beagle dogs, 8 males and 4 females, all young adults (1 to 3 years old), with an average weight of 10.6 kg ±1.42 were housed individually in twelve cages (1.83 m x 1.20 m) equipped with an acrylic-mesh floor and three-piece pan underneath to allow separation of feces and urine. The study was conducted as a replicated Latin square design (Kim and Stein, 2009). Each group of 3 animals was randomly assigned to a specific diet during each period, so that all dogs ate all diets by the conclusion of the study. The experiment lasted 56 days and it took place at the Large Animal Research Center (LARC) at Kansas State University, Manhattan, KS, U.S.A. There were four periods each divided into 9 days of adaptation and 5 days of sample collection. All animal testing was approved by the Kansas State University Institutional Animal Care and Use committee (IACUC) prior to the conduct of the study.

There were four nutritionally complete and balanced dietary treatments (Tables 3.1 and 3.2) fed to the dogs during the study: a rice, wheat and corn based control (CON), a whole sorghum based diet (WSD), a sorghum flour based diet (FLD), and a diet containing sorghum mill-feed (MFD) which was enriched with bran and some endosperm. All diets included titanium dioxide (0.4%) and chromium sesquioxide (0.25%) as markers. Diets were produced via extrusion processing (Chapter 2) to be similar in size, shape and texture at the Kansas State University laboratory. The food was stored in 9 kg poly-lined Kraft-paper bags.

Food allowances for dogs were intended to maintain body weight. As a starting point to determine dietary intake, daily metabolizable energy (ME) for each dog was calculated using the activity coefficients and empirical equations for laboratory, kennel housed dogs and (or) active pet dogs: $132 \times BW^{0.75}$ (BW=body weight; National Research Council; NRC, 2006). The finished

diet ME values were calculated using the modified Atwater values and empirical equation for ME in a dog food (AAFCO, 2016). From these data each dog food intake (in grams) was estimated. Daily diets were weighed according to these calculated amounts, and half given to dogs at 08:00 and 17:00. Based on dog weight, the food offered was adjusted throughout the study to maintain weight constant. Dogs had free access to water at all times and were housed in a climate-controlled building at 22-23°C.

During the collection period (day 9 to 14), dogs continued to be fed at 08:00 and 17:00 and any remaining orts were collected and weighed. Food offered and orts were weighed on a digital scale (model N1B110 Navigator, Ohaus Corporation, Switzerland) and recorded to determine total ingestion. All feces excreted during the 120 h of each collection period were harvested. Feces were scored for consistency, placed in plastic bags, and stored at -15°C until further analysis. The number of defecations were recorded each day and stools were scored using a 5 point scale (Table 4.3; Fig. 4.1), 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 scoring system was used with 0.5 point increments.

On the fifth day of the collection period, dogs were weighed, assessed for body condition score (BCS) and manually restrained for blood collection. Blood was drawn by brachial artery via venipuncture using 3 mL syringes and 22 g needles (BD medical technology, Franklin Lakes, NJ, U.S.A.). Approximately 3 mL of blood collected from each dog was immediately expelled into a test tube with ethylenediaminetetraacetic acid (EDTA) and placed on ice. At the culmination of blood collection the tubes were centrifuged at 2,000×g for 10 minutes to separate plasma. The plasma was harvested and frozen at -80°C for later analysis of antioxidant activity.

Nutrient Analysis

The nutrient composition of dietary treatments were determined at a commercial analytical laboratory (Midwest Laboratories, Omaha, Nebraska, U.S.A.). Methods of analysis included: moisture and dry matter (AOAC 930.15), organic matter and ash (AOAC 942.05), crude protein (AOAC 990.03), fat by acid hydrolysis (AOAC 954.02), crude fiber (AOCS Ba 6a-05), total starch (AACC 76-11; mod), starch gelatinized (AACC 76-11), and minerals calcium, phosphorus, potassium, magnesium, sodium, sulfur, copper, iron, manganese, and zinc (AOAC 985.01; mod).

Apparent Total Tract Digestibility Estimations

There were 4 techniques used to estimate fecal output (TFC, Cr_2O_3, TiO_2, AIA). These were each then used to calculate apparent total tract digestibility (ATTD). The ATTD using the total fecal collection (TFC) technique was calculated according to Eq. 3.1, and accounted for all feces excreted and food consumed during the collection period (5 days). The feces and food were dried in an electric oven (Cat 52755-20, Matheson Scientific, Morris Plains, NJ, U.S.A.) at 55°C until they were dry to the touch (24h-48h). This dry feces was further dried in the same oven at 155°C for 24h and values derived were used in the calculation of ATTD by TFC (AAFCO, 2016; 3.1). Food intake and feces excreted are both expressed on a dry matter basis.

$$\textit{Digestibility of nutrient} = \frac{\textit{(\% nutrient} \times \textit{total food intake)} - \textit{(\% nutrient} \times \textit{total feces)}}{\textit{(\% nutrient} \times \textit{total food intake)}} \qquad (3.1)$$

Chromic oxide was measured using the protocol described by Williams et al. (1962). Titanium dioxide was measured according to the protocol described by Myers et al. (2004). For both the titanium dioxide and chromic oxide determination, fecal and food samples were first ashed and then prepared for mineral analysis via atomic absorption, according to their respective

protocols. Acid insoluble ash in feces and food was determined using a slightly modified version of Keulen and Young (1977) protocol. Briefly, 4g of dry feces or 10g of food were weighed in porcelain crucibles on an analytical balance (Explorer: E1RW60, OHAUS, Parsippany, NJ, U.S.A.) and ashed at 600°C for 8h in a muffle furnace (model F30400: Thermo Scientific Thermolyne furnace, Asheville, NC, U.S.A.). These were then boiled in 2N hydrochloric acid (HCl) for 5 min before being filtered under vacuum (DOA-V120-AE: Gast Manufacturing, Inc., Bonton Harbor, MI, U.S.A.) through ashless filter paper (541: Whatman, Maidstone, United Kingdom), and then ashed again at 650°C overnight (approximately 12 h). The samples were weighed after the second ashing to estimate the AIA percentage. Apparent total tract digestibility was calculated with equation 3.2 (AAFCO, 2016), for all three markers (Cr_2O_3, TiO_2 and AIA):

$$\textit{Nutrient digestibility} = \left[1 - \frac{\textit{(\% marker in food} \times \textit{\% nutrient in feces)}}{\textit{(\% marker in feces} \times \textit{\% nutrient in food)}}\right] \times 100 \qquad (3.2)$$

Oxygen Radical Absorbance Capacity (ORAC)

An ORAC kit was purchased from Cell Biolabs, Inc. (San Diego, CA, U.S.A.). The chemicals included a fluorescein probe (100 ×), a free radical initiator in powder form (name not mentioned), a vitamin E antioxidant standard solution (Trolox™) of 5 mM, and assay diluent. All reagents were prepared and plasma samples were diluted 300-fold after several iterations to achieve the ideal dilutions. The procedure consisted of adding 25 uL of standard solutions (150, 100, 80, 60, 40, 20, 10, 5 and 2.5 Trolox™), blank solution, and samples in duplicate wells in a 96-well plate. To each well 150 uL of 1X fluorescein solution was added using a multi-channel pipette and then the plate was incubated at 37°C for 30 minutes. After incubation 25 uL of the free radical reagent initiator solution was added to each well and the plate was immediately inserted into the plate reader (Gen5™, Biotek® Instruments, Inc.Winooski, VT, U.S.A.) and

fluorescence was recorded every 1 min for 60 min at excitation and emission wavelengths of 480 and 520 nm, respectively. The results were calculated by the Gen5™ Microplate Data Collection and Analysis Software.

The area under the fluorescence decay curve (AUC) for each sample and standards were calculated using the equation below:

$$AUC = 2 + RFU_1/RFU_0 + RFU_2/RFU_0 + \ldots + RFU_{60}/RFU_0 \quad (3.3)$$

Where RFU_0= relative fluorescence value of time point zero and RFU_x= relative fluorescence value of time points. Next, Net AUC was calculated for all samples and standards using equation 3.4.

$$\text{Net AUC} = \text{AUC (antioxidant)} - \text{AUC (blank)} \quad (3.4)$$

Net AUC of standards was graphed on the y-axis against the Trolox™ Antioxidant Standard concentration on the x-axis. Micromole Trolox™ Equivalents (TE) of unknown samples were calculated by comparing samples' Net AUC to the standard curve. The sample concentrations were multiplied by their dilution (x300) and the results (ORAC values) were expressed as Trolox Equivalent (TE) per liter of sample.

Statistical Analysis

The experiment was conducted as a 4 X 4 replicated Latin Square design with 4 dietary treatments randomly assigned to 3 dogs during each of the 4 periods. Dogs were randomized to treatment and replicate by the procedure of Kim and Stein (2009). Throughout the course of the study all dogs received all treatments and served as their own controls. Fecal scores, number of defecations per day, feces excreted per day, food intake, nutrient digestibility of diets within each method (TFC, Cr_2O_3, TiO_2 and AIA), and ORAC value were evaluated with analysis of variance (ANOVA). Means were grouped using Bonferroni method by significant F values with $\alpha = 0.05$

of the generalized linear mixed model (GLIMMIX) procedure by statistical analysis software (SAS version 9.4; SAS Institute, Inc., Cary, NC). Correlation coefficients between digestibility methods (TFC, AIA, Cr_2O_3 and TiO_2) were determined with Multivariate analysis of variance (MANOVA) on 3 response variables: diet, period and dog, using the Generalized Linear Model (GLM) procedure with the aid of SAS (version 9.4) statistical software.

Results

Dog feeding study

The 4 dietary treatments were based on a similar level of protein, fat, minerals and vitamins, with slight adjustments due to their individual composition to the level of the carbohydrate sources (Table 3.1). The outcome (Table 3.2) would suggest that process drying was constant with an average final moisture of 5.92% and compared favorably to the target of 10% or less. However slight differences in crude protein and crude fat were noted. Specifically the crude protein level was slightly higher for the MFD dietary treatment. For crude fat the CON was 2.62% units greater ($P < 0.05$) than the MFD with WSD and FLD intermediate, but not different from the two extremes. The crude fiber was expected to differ by a greater proportion for the MFD diet in relation to the other treatments. There was a higher ($P < 0.05$) amount of starch in CON, WSD and FLD (average 47.5%) and lower in MFD (35.3%). From the starch present in the diets, FLD and MFD had greater ($P < 0.05$) gelatinization than CON and WSD. Minor difference in the minerals, magnesium and manganese might be artifacts of sampling.

Food intake by dogs throughout the 4 periods did not differ among treatments (average 187 g/d). Total feces collected were higher ($P < 0.05$) for dogs fed MFD (95.4 g/d), intermediate for dogs fed WSD (55.7 g/d) and lower for dogs fed CON and WSD diets (42.0 and 55.7 g/d,

respectively; Table 3.2). Defecations per day were more frequent ($P < 0.05$) when dogs were fed the MFD (3.02 times per day) and were similar for dogs fed CON, WSD and FLD (average 2.22 times per day). Average fecal score was higher for dogs fed MFD (3.92; $P < 0.05$) than CON (3.60), but did not differ from dogs fed WSD and FLD (3.68 and 3.78).

Digestibility Estimations

The ATTD for dogs fed MFD were generally lower than other treatments and ATTD for dogs fed the FLD were generally higher. Apparent total tract dry matter (DM) digestibility by total fecal collection (TFC) technique was higher ($P < 0.05$) for FLD and CON treatments (82.0% and 77.4%), intermediate for WSD (70.0%) and lowest for MFD treatment (51.0%; Table 3.4). Organic matter digestibility differed for all diets ($P < 0.05$): in increasing order they were 57.4%, 77.5%, 84.2% and 89.8% for MFD, WSD, CON and FLD, respectively. Energy, protein and fat digestibility were higher ($P < 0.05$) for dogs fed the CON and FLD, intermediate for the WSD treatment, and lower for those dogs fed MFD. The nitrogen free extract (NFE) digestibility, [which was calculated and not measured (NFE = DM-CP-CFi-CFa-Ash; AFFCO, 2016)] was greatest ($P < 0.05$) for dogs fed the FLD diet followed closely by CON. The WSD treatment had an NFE digestibility of 86.6% and the MFD treatment almost 20 percentage points lower (66.9%). Crude fiber digestibility was greater ($P < 0.05$) for WSD and FLD and lowest for CON and MFD treatments; but, in all treatments negative values suggest that more fiber was excreted than consumed. As observed for most of the other nutrients, most of the minerals were less ($P < 0.05$) utilized when dogs were fed the MFD and these followed closely behind the WSD dietary treatment, relative to dogs fed CON and FLD. Among the minerals potassium and sodium were the most highly utilized.

Apparent total tract digestibility calculated from fecal output estimated by use of Cr_2O_3 marker tended to overestimate digestibility values when compared to the TFC technique (Table 3.5). Dry matter digestibility differed for all treatments ($P < 0.05$), increasing from 65.9% to 81.1%, 83.0% and 86.0% for MFD, WSD, CON and FLD, respectively. Organic matter digestibility was higher ($P < 0.05$) for dogs fed FLD (90.7%), intermediate for the CON and WSD treatments (88.0% and 85.8%) and lowest for the MFD treatment (70.6%). Energy and protein digestibility calculated using Cr_2O_3 were higher for FLD, intermediate for CON and WSD and lower for MFD. Fat digestibility was higher ($P < 0.05$) for CON and FLD, intermediate for WSD and lowest for dogs fed MFD. Nitrogen free extract (NFE) ATTD differed in order ($P < 0.05$) of FLD> CON> WSD and MFD, with the 3 first treatments exceeding 90% and MFD slightly below 80.0%. Similar to estimates by TFC, the crude fiber digestibility was also lowest ($P < 0.05$) for CON and MFD and greatest for WSD and FLD treatments. The mineral digestibilities were different ($P < 0.05$) among the treatments and the MFD tended to result in lowest values. However, by Cr_2O_3 analysis the mineral digestibilities were numerically greater when compared to digestibilities estimated by TFC.

Dry matter, organic matter and energy digestibility calculated using TiO_2 estimates of fecal output followed a similiar pattern to Cr_2O_3; wherein, digestibility by dogs fed FLD was higher ($P < 0.05$) than CON, which was higher than WSD, and in turn higher than MFD (Table 3.6). Crude protein digestibility was higher for FLD (80.3%), intermediate for CON and WSD (77.0% and 76.0%) and lower for the MFD treatment (66.3%). Crude fat digestibility measured by titanium dioxide followed the same order as that measured by cromic oxide; wherein, CON and FLD were higher, WSD intermediate and MFD was lower. The NFE ATTD digestibility was very similar to the chromic oxide technique; wherein, there was a numerical decreasing order for

FLD, CON, and WSD with above 90%, and MFD lowest below 80%. Similarly to TFC and Cr_2O_3 techniques, fiber digestibility was lowest ($P < 0.05$) for CON and MFD and highest for WSD and FLD treatments. Mineral digestibilities were, in general, lower ($P < 0.05$) for MFD treatment, and aligned similar with results observed from the Cr_2O_3 technique.

Dry matter, organic matter, energy and crude protein digestibility estimated by AIA, as the fecal output marker, all followed the same trend ($P < 0.05$); wherein, FLD had the highest values, CON and WSD were intermediate, and MFD had the lowest digestibility values (Table 3.7). Crude fat digestibility was higher ($P < 0.05$) for FLD, intermediate for WSD, lower for MFD, and CON was similar to FLD. Nitrogen free extract (NFE) ATTD by AIA marker was closer to the TFC technique than the other markers, but all the techniques resulted in the same decreasing order of FLD, CON, WSD and MFD. Crude fiber digestibility was lowest ($P < 0.05$) for CON, intermediate for MFD and highest for WSD and FLD. The mineral digestibilities differed ($P < 0.05$) among treatments, and similar to the other fecal output techniques they tended to be lower for MFD.

The fecal output of dry matter (DM), organic matter (OM), crude protein (CP), crude fat (CFa) and crude fiber (CFi) were compared by the partial correlation coefficients from the Error SSCP Matrix (Table 3.8-3.13). The TFC technique had the lowest correlations for all nutrients tested, but still had a significant correlation ($P < 0.05$) with TiO_2 and Cr_2O_3, which ranged from 0.50 to 0.82. Crude fiber (CFi) was the only fecal output of a nutrient that TFC correlated with ($P < 0.05$; 0.54; Table 3.12) relative to the other estimates of fecal output. Interestingly, TiO_2 and Cr_2O_3 had a very high correlation ($P < 0.05$; average R=0.93) for all fecal output estimates of the nutrients, with an average of 0.93. The estimates of fecal output using AIA as the marker correlated better with TiO_2 (average 0.83) and secondly to Cr_2O_3 (average 0.75). It was expected

that Cr_2O_3 as the standard marker for dogs (AFFCO; 2015b) would correlate better to the TFC technique than TiO_2.

The plasma ORAC value in dogs fed the MFD was over 2-fold higher ($P < 0.05$) than each of the other treatments CON, FLD and WSD (20,482 vs average 8,923 μM TE/L). It was expected dogs fed WSD would have higher plasma ORAC values, but they did not differ from CON and FLD.

Discussion

Conducting feeding trials with the target species is essential when developing a new dietary product. Parameters such as acceptability, stool consistency, nutrient utilization and overall healthfulness can be assessed. Assessment of nutrient utilization can be quantified by determination of fecal output. This can be done by collecting all feces or through use of a marker. Chromium sesquioxide (Cr_2O_3) is a common marker used to calculate digestibility and it is to date the official indicator method for determination of metabolizable energy in dog and cat foods specified by AAFCO (2016). However, Cr_2O_3 has had poor repeatability among laboratories and may be hazardous, according to the review of Sales and Janssens (2003). Alternative markers like titanium dioxide (TiO_2), an external marker, or acid insoluble ash (AIA), an internal marker, might prove beneficial to companion animal nutrition research.

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 intake by the dogs was not different among the treatments in this study (average 186.8 g/d; Table 3.3), suggesting that the dietary treatments did not influence voluntary intake or acceptability. Likewise, Kore et al. (2009) and Teixeira (2015) also reported that diets containing sorghum did not influence acceptance by dogs. The amount of wet

feces excreted was greatest for MFD at nearly 3× that of FLD. Likewise, the number of defecations per day was more frequent for dogs fed 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 scores (3.92 on a 5 point scale in which 4 is firm dry feces). The same occurrence was observed in a study of Teixeira (2015) in which dogs fed diets that contained sorghum produced larger quantities of feces, but the fecal scores were also not affected (Teixeira, 2015). Similar to our study, the author observed that dogs fed sorghum-containing diets produced less fetid (smelly) stool, although this observation was worker observation only and not quantified. In other studies, fecal scores of dogs that were fed a sorghum based diet were similar to dogs fed a corn-based diet (Twomey et al., 2012; Kore et al., 2009). In a nutrition study with humans, Fedail et al. (1984) found that sorghum bran increased stool weight, number of defecations and decreased intestinal transit time. This mirrors what was observed for the Beagle dogs in our study fed the MFD. Burrows et al. (1982) also reported that fiber addition decreased transit time and increased fecal bulk in dogs; more specifically, a 6% cellulose addition had a similar fecal weight per day as the MFD. Sorghum is low in soluble fiber and rich in insoluble fiber (Bach Knudsen and Munck, 1985), which is more concentrated in the bran fraction. This factor likely led to the increased stool bulk and number of defecations that were observed.

In our study, dry matter (DM) and organic matter (OM) digestibility of dogs fed WSD and MFD by TFC technique were lower than what was expected. A study evaluating the effects of fiber on apparent digestibility in Beagle dogs reported a decrease of 2.2% in the DM apparent digestibility for every percent of added cellulose (Burrows et al., 1982), which is an insoluble fiber similar to what is present in sorghum bran. Kore et al. (2009) reported that dogs fed a diet

containing 70% whole sorghum had a total tract DM and OM apparent digestibility of 83.1 and 85.1%, respectively, and were similar values to animals fed a rice or corn-based diet. Carciofi et al. (2008) also reported that the DM and OM apparent total tract digestibility of dogs fed a diet containing almost 60% sorghum were close to 80%, and similar to results from a corn based diet. Surprisingly, in our study the DM digestibility of dogs fed the CON treatment was 7.4 percentage points greater than those fed the WSD. The FLD had the highest DM and OM digestibility, and compared favorably to the digestibility of a cassava flour based diet reported by Carciofi et al. (2008). It was anticipated that dogs fed the MFD would have lower digestibility values, but a DM and OM digestibility of 51.0% and 57.4% were less than expected.

All the diets from the present study had low crude protein (CP) digestibility values. According to the TFC technique, The CP digestibility of WSD treatment was 7.8 percentage units lower than that of CON and the CP digestibility of MFD treatment was 17.8 percentage units lower than CON, a large difference. Our findings disagree with some previous studies conducted with dogs (Kore et al., 2009; Carciofi et al., 2009; Twomey et al., 2002). For instance, Kore et al. (2009) reported a protein digestibility of sorghum-based diet to be over 90% and similar to diets containing rice and maize as their only starch source. Carciofi et al. (2008) reported that the apparent total tract CP digestibility of a sorghum-based diet was 85.0%, which was similar to a cassava flour, corn and pea-based diets, and greater than a lentil-based diet. Twomey et al. (2002) also found ATTD of a sorghum-based diet to be similar to corn-based diets. Conversely, Teixeira (2015) reported that diets with sorghum negatively influence ATTD by TFC technique for DM, OM, CP, CF, digestible energy and metabolizable energy for dogs. Kurien et al. (1960) replaced rice with sorghum in a vegetarian diet for healthy boys and reported a decrease in protein digestibility from 75 to 55%. Similarly, the sorghum variety used in our

study to produce WSD and MFD also led to lower protein digestibility in dogs. This could be related to some polyphenols that influenced ATTD. Also, in high fiber diets the fermentation of fiber generates additional bacterial protein in the feces which may account for extra fecal nitrogen and thereby underestimate apparent total tract protein digestibility (Gross et al., 2000).

Sorghum proteins have a similar or lower *in vitro* digestibility than other cereals (Hamaker and Bugusu, 2003). The reasons for this have not fully been elucidated, but factors that may contribute to lower digestibility include the physical grain structure, protein cross-linking, starch properties, and phenolic compounds of the grain that can complex with both protein and carbohydrates and generate insoluble compounds resistant to digestion (Lemlioglu, 2014). In the present study digestibility of sorghum proteins were of minor importance since the main source of amino acids were the poultry meal and corn gluten meal. However, ATTD of crude protein in the MFD may have declined due to interactions with polyphenols present in the sorghum bran. Condensed tannins (CT) chelate iron and complex with proteins through the formation of hydrogen bonds and hydrophobic interactions (Scalbert et al., 2000). Kaufman et al. (2013) studied the effects of CT on protein digestibility and found that CT content negatively affected bovine serum albumin digestibility by binding to the protein. These authors also found that differences in CT molecular weight distribution had a significant impact on protein digestibility. This may mean that a low CT sorghum could lower protein digestibility if it consisted mainly of the very active molecular weight range tannins. *In vivo* CT effect on digestibility may not be drastic if ingested in moderate amounts. Condensed tannins are only absorbed when broken down into smaller molecules and they bind non-specifically to proteins (stronger binding when high weight tannins) and would be unlikely to influence proteins biological properties (Scalbert et al., 2000).

Nitrogen free extract (NFE) apparent total tract digestibility was low for WSD and much lower for the MFD treatment. An optimal starch digestibility should not be a concern for dogs and cats in healthy conditions since carbohydrates are not an essential nutrient. It is common that people overfeed their pets. In the US, around 17.6% of dogs are obese and 35.1% are overweight (APOP, 2014). Amoako et al. (2016) investigated the effect of CT sorghum on starch and found that polymeric CT interact with starch and this interaction may be enhanced in high amylose-containing starch due to the more extensive hydrogen bonding and hydrophobic interactions. In essence lower starch digestibility may actually be beneficial under certain circumstances. Sorghum bran is the fraction with highest proanthocyanidin content (Awika et al., 2005). Besides the interactions between proteins and polyphenols relative strength when dealing with high molecular weight CT, sorghum proanthocyanidins have also been found to interact with starch molecules to a greater extent than shorter-chain phenolics (Barros et al., 2012). These interactions may help explain the lower digestibility values.

The crude fiber (CFi) digestibility results were difficult to conclusively summarize. The CON and MFD had negative ATTD values by all techniques used which means that there was more crude fiber excreted than consumed. Carciofi et al. (2008) reported an average crude fiber ATTD of 3.5% for diets containing different types of starch sources. Fahey et al. (1990) also reported low CFi digestibility values for dogs fed diets with different concentrations of beet pulp, but they were greater than what was found in our study (average 24%). Similarly, Teixeira (2015) reported a crude fiber ATTD by TFC technique to range between 31.0% and 35.1% for dogs fed sorghum-based diets. These low values for crude fiber digestibility suggest that the material is relatively indigestible and may not provide meaningful information for which to draw conclusions. The ash digestion may have some value as a gross indication of overall mineral

utilization and tended to be lower for dogs fed the MFD treatment. Most minerals are utilized according to the animal needs (i.e. vitamin D3 controls absorption of calcium), so digestibility values are hard to interpret short of a gross swing due to treatment. In the present study the electrolytes potassium and sodium disappearance seemed to have been reduced in CON, WSD and FLD fed dogs related to those fed MFD. Other researchers have reported varying mineral absorption with large variation and some negative values (Lewis et al., 1994; Baynen et al. 2002). The polyphenols chelate iron (Hurrel et al., 1999, Kennedy and Powell, 1985; Yoshino and Muramaki, 1998) and this may have happened in the present study; wherein, dogs fed the MFD had the lowest iron disappearance regardless of which marker technique was used.

Markers to estimate fecal output used to calculate digestibility tended to overestimate digestibility values in comparison to the TFC technique. The use of markers to estimate fecal output allows for feces quantification in semi-confining free roaming animals and decreases time required to plan and execute collection. Further, it is not practical to expect that all feces be collected because some animals may be coprophagic, feces may get mixed with urine, or stepped on by animals in confined spaces. All of which occurred in this study and lead us to believe fecal output by TFC may have underestimated excretion.

The Pearson correlation coefficients for fecal output using markers or TFC yielded some unexpected results. Titanium dioxide seemed to perform best with the lowest variation (SEM; Table 3.5). This marker correlated well with Cr_2O_3 for DM, OM, CP, CFa and energy, and it also had the highest correlation to AIA and TFC. Vasconcellos et al. (2007) used TFC, lignin, Cr_2O_3 and AIA as markers to estimate fecal output and calculate ATTD of DM, CP, CFa, OM and energy of cats fed 4 diets formulated with different carbohydrate sources (corn, sorghum, tapioca flour or brewers rice). The authors found that results from TFC, Cr_2O_3 and AIA for all nutrients

and diets did not differ. Hill et al. (2009) reported similar apparent nutrient digestibility coefficients between TFC and TiO_2 techniques for dogs. Kavanagh et al. (2001) compared Cr_2O_3, TiO_2, TFC and AIA techniques to determine apparent digestibility in growing pigs. They reported that DM and energy apparent digestibility were greater ($P < 0.05$) when using the techniques TFC, AIA and Cr_2O_3, and lower when using TiO_2 or Cr_2O_3 as markers. Titanium dioxide is also a common food additive used to color (whiten) foods and is allowed by the Code of Federal Regulations (CFR, 2015) up to 1%.

There was additional benefit to AIA as an option because it is intrinsic to the diet and does not need to be added. Especially in pet diets because the ash content of the food resulting from bone minerals found in common rendered protein meals is typically sufficient. There are at least 3 procedures reported that can be used to measure AIA in food and feces (Keulen and Young, 1977). In the present study it was noted that the protocol which specified 2 ashings and used 2N HCl seemed to work best. This is because boiling samples in acid prior to ashing created a black and fetid substance that adhered to the beakers and resulted in incomplete recovery. Although not the highest correlation among the methods, acid insoluble ash (AIA) compared well to Cr_2O_3 and TiO_2 for all parameters. This is encouraging because AIA may have beneficial applications for animals that cannot be fed prepared diets with added external markers. But AIA did have a poor correlation with TFC. A study conducted with dogs found that both AIA and Cr_2O_3 were highly correlated with TFC (Lobo-Junior et al., 2001). Zanatta et al. (2013) used the same protocol for AIA that was used in the present study and they reported a high correlation between AIA and TFC techniques to estimate fecal output and nutrient digestibilities in dogs. Our concern was that TFC was not reflective of true fecal output and may have led to the inconsistent interpretation. Thus, there is need to further explore the use of AIA as an intrinsic

fecal marker for dogs. Based on the results, titanium dioxide appeared to be the marker of choice.

While the condensed tannins may reduce nutrient digestibility some, there are benefits to polyphenol consumption by animals. The pet market is concerned with animal health and longevity and there are several studies reporting the antioxidant impact of phenolic compounds found in the pericarp of most sorghum cultivars (Awika and Rooney, 2004; Moraes et al., 2015; Awika et al., 2003b; Ishimoto et al., 2012; Kaufman et al., 2013). In our study, plasma from dogs that consumed the MFD had more than twice the ORAC value of WSD, FLD and CON (table 3.7). It was expected that the WSD would also lead to a higher antioxidant capacity, but this was not observed. Moraes et al. (2015) found that the antioxidant activity of extracts from sorghum fractions measured *in vitro* by ORAC was lower in decorticated sorghum flour, five times greater in whole sorghum flour, and 10 times greater in sorghum bran. The ORAC assay was chosen for their experiment because it used biologically relevant free radicals and integrated both time and degree of inhibition according to Awika et al. (2003b). There is no work that previously evaluated dog plasma antioxidant activity after consumption of diets formulated with sorghum fractions.

Based on this work pet food companies could 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. Polymeric proanthocyanidins can naturally modify starch by interacting strongly with amylose (Barros et al., 2012). 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.

Summary

The diets produced with sorghum fractions were well accepted by dogs, resulted in satisfactory fecal scores, albeit with higher fecal mass and number of defecations for MFD. The MFD had the lowest digestibility values and this may be due to its higher fiber content, and possible CT interactions with some nutrients. The marker that best correlated with Cr_2O_3 to estimate fecal output of the nutrients tested was TiO_2, followed by AIA. Titanium dioxide had higher correlation to all markers and it can be used as a food additive up to 1%. According to the present study it would be preferred over Cr_2O_3. There is a need to further explore the use of AIA as an intrinsic marker for estimating fecal output when addition of a marker into the diet is not possible. The sorghum flour may have an application in easy-to-digest foods for dogs and cats with specific health conditions, while sorghum bran may be included in diets for obese and diabetic pets. Further, sorghum bran can be used in a certain proportion in diets to provide antioxidant capacity for pets.

Table 3.1 Experimental diets used to feed dogs: Control (CON), whole sorghum (WSD), sorghum flour (FLD) and sorghum mill-feed (MFD).

Ingredients, %	CON	WSD	FLD	MFD
Brewers rice	21.2	-	-	-
Corn	21.2	-	-	-
Wheat	21.2	-	-	-
Whole sorghum	-	64.7	-	-
Sorghum flour	-	-	62.3	-
Sorghum mill-feed	-	-	-	67.6
Chicken by-product meal	20.9	20.0	21.0	20.0
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 premix[a]	0.15	0.15	0.15	0.15
Trace mineral premix[b]	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

[a]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.
[b]Trace mineral premix: calcium carbonate, zinc sulfate, ferrous sulfate, copper sulfate, manganous oxide, sodium selenite, and calcium iodate.

Table 3.2 Nutrient analysis of experimental diets control (CON), whole sorghum (WSD), sorghum flour (FLD) and sorghum mill-feed (MFD) used during dog feeding study (N=2). Means were separated by Tukey grouping.

Nutrient	CON	WSD	FLD	MFD	SEM	P
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	0.7569	0.8937
Crude protein, %	21.5[ab]	21.4[ab]	21.2[b]	23.8[a]	0.434	0.0355
Crude fat, %	12.10[a]	10.7[ab]	10.25[ab]	9.48[b]	0.392	0.0373
NFE (calculated), %	52.4[b]	53.6[ab]	55.9[a]	51.2[b]	0.67	0.0081
Crude fiber, %	0.675[b]	1.330[ab]	0.725[b]	2.710[a]	0.330	0.0346
Total starch, %	46.9[a]	45.6[a]	50.0[a]	35.3[b]	1.71	0.0140
Starch gelatinized, %	85.2[b]	86.1[b]	96.3[a]	93.5[a]	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
Potassium, %	0.55	0.66	0.62	0.60	0.0265	0.0845
Sodium, %	0.290	0.290	0.275	0.250	0.0090	0.0936
Magnesium, %	0.095[b]	0.135[ab]	0.100[b]	0.165[a]	0.0083	0.0113
Iron, ppm	179.0	180.5	161.5	187.0	7.06	0.2102
Copper, ppm	19.2	17.0	15.8	18.3	2.105	0.7023
Zinc, ppm	143.5	159.5	136.5	165.5	15.548	0.5737
Manganese, ppm	27.8[ab]	25.2[ab]	19.6[b]	34.0[a]	2.24	0.0443

[ab]Means within a row that lack a common superscript differ ($P \leq 0.05$).

Table 3.3 Food intake and feces collected (on dry matter basis) per day, number of defecations per day and fecal scores of dogs fed control (CON), whole sorghum (WSD), flour (FLD) diets and mill-feed (MFD) diets (N=12). Means were separated by Bonferroni grouping.

Item	CON	WSD	FLD	MFD	SEM	P
Food intake, g/d	185	186	181	195	6.5	0.4818
Feces excreted, g/d	42.0[c]	55.7[b]	32.6[c]	95.4[a]	3.24	<.0001
Defecations per day	2.18[b]	2.38[b]	2.10[b]	3.02[a]	0.098	<.0001
Fecal score	3.60[b]	3.68[ab]	3.78[ab]	3.92[a]	0.068	0.0007

[abc] Means within a row that lack a common superscript differ ($P \leq 0.05$).

Figure 3.1 Five-point fecal scoring chart used to score dog feces that were fed control (CON), whole sorghum (WSD), sorghum flour (FLD) and sorghum mill-feed (MFD) diets.

1	2	3	4	5
				
watery, liquid diarrhea	soft, unformed stool	softer stool, retains shape	Firm, formed stool (ideal)	very hard, dry pellets

Table 3.4 Apparent total tract digestibility determined by estimates of fecal output by total fecal collection (TFC) of dogs fed control (CON), whole sorghum (WSD), flour (FLD) and mill feed (MFD) diets (N=12). Means were separated by Bonferroni grouping.

Item	CON	WSD	FLD	MFD	SEM	P
Dry Matter, %	77.4[a]	70.0[b]	82.0[a]	51.0[c]	1.26	<.0001
Organic Matter, %	84.2[b]	77.5[c]	89.8[a]	57.4[d]	1.01	<.0001
Energy, %	83.1[a]	75.8[b]	87.6[a]	56.8[c]	1.13	<.0001
Crude Protein, %	70.2[a]	62.4[b]	76.9[a]	52.4[c]	1.70	0.0001
Crude Fat, %	88.8[a]	81.5[b]	89.0[a]	67.8[c]	1.04	0.0001
NFE (calculated), %	92.3[b]	86.6[c]	95.3[a]	66.9[d]	0.61	<.0001
Crude Fiber, %	-128.7[b]	-18.2[a]	-10.8[a]	-89.1[b]	11.27	<.0001
Ash, %	3.12[a]	-31.38[c]	-2.35[ba]	-15.35[b]	4.691	<.0001
Calcium, %	-44.0[ab]	-56.9[b]	-33.8[a]	-60.8[b]	6.24	0.0147
Phosphorus, %	-20.67[ab]	-31.06[bc]	-9.48[a]	-45.10[c]	5.37	0.0002
Potassium, %	89.9[b]	85.4[c]	94.2[a]	79.2[d]	1.40	0.0001
Sodium, %	89.8[a]	83.1[b]	92.4[a]	29.3[c]	2.14	0.0001
Magnesium, %	-8.57[a]	-29.62[b]	-6.14[a]	-55.24[c]	5.455	0.0001
Iron, %	-12.10[a]	-41.23[b]	-1.26[a]	-80.12[c]	6.3893	0.0001
Copper, %	-26.2[ab]	-44.6[b]	-24.9[a]	-71.5[c]	6.87	0.0001
Zinc, %	-48.2[b]	-63.3[c]	-21.5[a]	-74.3[c]	7.14	0.0001
Manganese, %	-7.18[a]	-69.88[c]	-40.91[b]	-85.06[c]	6.416	0.0001

[abcd] Means within a row that lack a common superscript differ ($P \leq 0.05$).

Table 3.5 Apparent total tract digestibility determined by estimates of fecal output using chromic oxide as an external marker of dogs fed control (CON), whole sorghum (WSD), flour (FLD) and mill feed (MFD) diets (N=12). Treatments means were separated by Bonferroni grouping.

Item	CON	WSD	FLD	MFD	SEM	P
Dry Matter, %	83.0[b]	81.1[c]	86.0[a]	65.9[d]	0.44	<.0001
Organic Matter, %	88.0[b]	85.8[b]	90.7[a]	70.6[c]	0.34	<.0001
Energy, %	87.2[b]	85.4[b]	90.3[a]	70.2[c]	0.68	<.0001
Crude Protein, %	77.5[b]	76.3[b]	81.8[a]	67.2[c]	0.73	<.0001
Crude Fat, %	91.5[a]	88.4[b]	91.4[a]	77.9[c]	0.37	<.0001
NFE (calculated), %	94.2[b]	91.6[c]	96.3[a]	77.0[d]	0.35	<.0001
Crude Fiber, %	-75.2[c]	24.7[a]	12.3[a]	-29.7[b]	7.25	<.0001
Ash, %	26.6[a]	17.5[b]	19.2[b]	11.8[c]	1.78	<.0001
Calcium, %	-9.05[b]	1.33[a]	-5.74[b]	-11.10[b]	2.294	0.0023
Phosphorus, %	8.65[b]	17.66[a]	13.55[ba]	-0.25[c]	1.913	0.0010
Potassium, %	92.4[b]	92.3[b]	95.4[a]	85.6[c]	0.81	<.0001
Sodium, %	92.4[ba]	89.4[b]	94.1[a]	51.0[c]	1.19	<.0001
Magnesium, %	17.6[a]	18.6[a]	16.2[a]	-7.3[b]	2.165	<.0001
Iron, %	14.9[a]	11.2[a]	19.8[a]	-24.8[b]	3.22	<.0001
Copper, %	4.48[ba]	9.37[a]	1.32[b]	-18.36[c]	2.797	<.0001
Zinc, %	-8.59[b]	-1.91[ba]	4.22[a]	-20.36[c]	2.439	<.0001
Manganese, %	18.94[a]	-6.68[b]	-11.46[b]	-27.86[c]	2.316	<.0001

[abcd] Means within a row that lack a common superscript differ ($P \leq 0.05$).

Table 3.6 Apparent total tract digestibility determined by estimates of fecal output using titanium dioxide as an external marker of dogs fed control (CON), whole sorghum (WSD), flour (FLD) and mill feed (MFD) diets (N=12). Treatment means were separated by Bonferroni grouping.

Item	CON	WSD	FLD	MFD	SEM	P
Dry Matter, %	82.7[b]	80.8[c]	84.9[a]	65.0[d]	0.26	<.0001
Organic Matter, %	87.8[b]	85.6[c]	90.0[a]	69.8[d]	0.25	<.0001
Energy, %	86.9[b]	84.6[c]	89.4[a]	69.4[d]	0.32	<.0001
Crude Protein, %	77.0[b]	76.0[b]	80.3[a]	66.3[c]	0.59	<.0001
Crude Fat, %	91.3[a]	88.2[b]	90.6[a]	77.3[c]	0.35	<.0001
NFE (calculated), %	94.1[b]	91.4[c]	96.0[a]	76.3[d]	0.36	<.0001
Crude Fiber, %	-78.23[c]	25.03[a]	4.86[a]	-33.15[b]	6.679	<.0001
Ash, %	25.1[a]	16.2[b]	12.5[c]	9.4[d]	0.85	<.0001
Calcium, %	-11.3[b]	-0.2[a]	-14.5[b]	-14.1[b]	1.37	<.0001
Phosphorus, %	6.72[b]	16.18[a]	6.36[b]	-2.92[c]	1.362	0.0010
Potassium, %	92.2[b]	92.3[b]	95.1[a]	85.2[c]	0.78	<.0001
Sodium, %	92.2[ba]	89.2[b]	93.6[a]	49.7[c]	1.22	<.0001
Magnesium, %	15.9[a]	17.3[a]	9.2[b]	-10.2[c]	1.61	<.0001
Iron, %	13.1[a]	10.1[a]	13.0[a]	-28.1[b]	2.83	<.0001
Copper, %	2.38[a]	7.75[a]	-6.75[b]	-21.58[c]	2.452	<.0001
Zinc, %	-10.77[b]	-3.81[a]	-3.73[a]	-23.64	1.685	<.0001
Manganese, %	17.2[a]	-8.5[b]	-20.7[c]	-31.3[d]	1.43	<.0001

[abcd] Means within a row that lack a common superscript differ ($P \leq 0.05$).

Table 3.7 Apparent total tract digestibility determined by AIA of dogs fed control (CON), whole sorghum (WSD), flour (FLD) and mill feed (MFD) diets (N=12). Treatments means were compared by Bonferroni grouping.

Item	CON	WSD	FLD	MFD	SEM	P
Dry Matter, %	76.4[b]	77.0[b]	85.0[a]	50.4[c]	1.09	<.0001
Organic Matter, %	83.3[b]	82.7[b]	90.5[a]	57.1[c]	0.98	<.0001
Energy, %	82.2[b]	81.5[b]	90.1[a]	56.5[c]	1.08	<.0001
Crude Protein, %	68.5[b]	71.1[b]	81.4[a]	52.4[c]	1.31	<.0001
Crude Fat, %	88.2[ab]	85.9[b]	91.2[a]	67.8[c]	0.83	<.0001
NFE (calculated), %	92.0[b]	89.7[b]	96.2[a]	66.4[c]	0.92	<.0001
Crude Fiber, %	-143.1[c]	10.2[a]	10.2[a]	-87.7[b]	9.06	<.0001
Total starch, %	46.9[a]	45.6[a]	50.0[a]	35.3[b]	1.71	0.0140
Starch gelatinized, %	85.2[b]	86.1[b]	96.3[a]	93.5[a]	1.28	0.0084
Ash, %	-2.25[b]	-0.59[b]	17.44[a]	-28.19[c]	3.194	<.0001
Calcium, %	-51.9[c]	-19.95[b]	-8.07[a]	-61.61[c]	4.036	<.0001
Phosphorus, %	-27.09[c]	-0.13[b]	11.63[a]	-45.52[d]	3.164	<.0001
Potassium, %	89.3[b]	90.6[b]	95.4[a]	79.2[c]	1.12	<.0001
Sodium, %	89.3[a]	87.0[a]	94.0[a]	28.3[b]	2.62	<.0001
Magnesium, %	-14.55[c]	0.87[b]	14.34[a]	-55.90[d]	3.765	<.0001
Iron, %	-18.33[b]	-7.31[b]	17.92[a]	-81.67[c]	5.14	<.0001
Copper, %	-32.97[b]	-10.38[a]	-0.75[a]	-71.84[c]	4.493	<.0001
Zinc, %	-51.0[c]	-24.4[b]	2.10[a]	-75.0[d]	4.36	<.0001
Manganese, %	-12.9[a]	-29.8[b]	-13.9[a]	-85.8[c]	4.299	<.0001

[abc] Means within a row that lack a common superscript differ ($P \leq 0.05$).

Table 3.8 Partial Correlation Coefficients from the Error SSCP Matrix* (Pearson) evaluating methods to determine dry matter (DM) fecal output by dogs in which dietary treatment data were pooled.**

	TFC	Cr_2O_3	TiO_2	AIA
TFC	-	0.50*	0.55*	0.22
Cr_2O_3	-	-	0.91*	0.71*
TiO_2	-	-	-	0.82*

* Coefficient of correlation significant at $P < 0.05$.
**total fecal collection (TFC); chromic oxide (Cr_2O_3); titanium dioxide (TiO_2); acid insoluble ash (AIA).

Table 3.9 Partial Correlation Coefficients from the Error SSCP Matrix* (Pearson) evaluating methods to determine organic matter (OM) fecal output by dogs in which dietary treatment data were pooled.**

	TFC	Cr_2O_3	TiO_2	AIA
TFC	-	0.56*	0.59*	0.29
Cr_2O_3	-	-	0.93*	0.75*
TiO_2	-	-	-	0.84*

* Coefficient of correlation significant at $P < 0.05$.
**total fecal collection (TFC); chromic oxide (Cr_2O_3); titanium dioxide (TiO_2); acid insoluble ash (AIA).

Table 3.10 Partial Correlation Coefficients from the Error SSCP Matrix (Pearson) of methods used to determine crude protein (CP) dog fecal output.

	TFC	Cr_2O_3	TiO_2	AIA
TFC	-	0.57*	0.61*	0.33
Cr_2O_3	-	-	0.91*	0.73*
TiO_2	-	-	-	0.82*

* Coefficient of correlation significant at $P < 0.05$.
**total fecal collection (TFC); chromic oxide (Cr_2O_3); titanium dioxide (TiO_2); acid insoluble ash (AIA).

Table 3.11 Partial Correlation Coefficients from the Error SSCP Matrix (Pearson) of methods used to determine crude fat (CFa) dog fecal output.

	TFC	Cr_2O_3	TiO_2	AIA
TFC	-	0.57*	0.61*	0.33
Cr_2O_3	-	-	0.91*	0.73*
TiO_2	-	-	-	0.82*

* Coefficient of correlation significant at $P < 0.05$.
**total fecal collection (TFC); chromic oxide (Cr_2O_3); titanium dioxide (TiO_2); acid insoluble ash (AIA).

Table 3.12 Partial Correlation Coefficients from the Error SSCP Matrix (Pearson) of methods used to determine crude fiber (CFi) dog fecal output.

	TFC	Cr_2O_3	TiO_2	AIA
TFC	-	0.81*	0.82*	0.54*
Cr_2O_3	-	-	0.99*	0.79*
TiO_2	-	-	-	0.84*

* Coefficient of correlation significant at $P < 0.05$.
**total fecal collection (TFC); chromic oxide (Cr_2O_3); titanium dioxide (TiO_2); acid insoluble ash (AIA).

Table 3.13 Partial Correlation Coefficients from the Error SSCP Matrix (Pearson) of methods used to determine Energy dog fecal output.

	TFC	Cr_2O_3	TiO_2	AIA
TFC	-	0.56*	0.60*	0.33
Cr_2O_3	-	-	0.93*	0.78*
TiO_2	-	-	-	0.86*

* Coefficient of correlation significant at $P < 0.05$.
**total fecal collection (TFC); chromic oxide (Cr_2O_3); titanium dioxide (TiO_2); acid insoluble ash (AIA).

Table 3.14 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).

Item	CON	WSD	FLD	MFD	SEM	P
ORAC value, μM TE/L	9,563[b]	8,621[b]	8,584[b]	20,482[a]	1313.5	<.0001

TE= Trolox equivalent.
[ab]Means within a row that lack a common superscript differ ($P \leq 0.05$).
Treatments means were compared by Bonferroni grouping.

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Appendix A - Flour mill settings to produce sorghum fractions

Table A.1 Flour mill settings used to produce sorghum fractions.

Mill:	KANSAS STATE UNIVERSITY																Location:	MANHATTAN, KS / USA						Corrugation Table No.:						
Capacity:	397	cwt./24h															Flowsheet #:						Date:		Comments:					
Rollermill		Grinding Rolls							Flutes								Smooth roll	Feed rolls						Transmission		Drive				
Type	Passage	Number	Length (mm)	Length (in)	Diameter (mm)	Thick-walled	Aut. roll gap adj.	Watercooling	Flutes per circum.	Flutes per cm	Flutes per in.	Grinding action	Style of grooves	Spiral %	Land / mm	Land / in.	Tappering / µ (on diameter)	Type front	Type back	Speed front	Speed back	Distribution Bridge	Tooth belt ratio	Scrapper type	rpm	Motor / kW	Motor / HP	kW / t	HP / cwt.
MDDO	1BK	2	800	31.50	250				350	4.5	11.3	D	18	2	0.2			S2	-				1 : 2.5	-	350	11.0	15	0.458	0.037
MDDO	2BK	2	800	31.50	250				450	5.7	14.6	D	18	4	0.2								1 : 2.5	-	350				
MDDM	2BK	2	800	31.50	250				450	5.7	14.6	D	18	4	0.2			PT5	W				1 : 2.5	B	350	7.5	10	0.313	0.025
MDDM	3BK/D1	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	0.025
MDDM	4BK	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	0.025
MDDM	5BK	2	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	0.025
MDDM	1.SIZ/D2	2	500	19.69	250				850	10.8	27.5	A	18	6									1 : 2.5	B	350	7.5	10	0.313	0.025
MDDM	2.SIZ/D3	2	500	19.69	250				900	11.5	29.1	A	18	6									1 : 2.5	B	350				
MDDO	1M	2	800	31.50	250				smooth								30 - 35					X	1 : 1.23	S	220	11.0	15	0.458	0.037
MDDO	2M	2	800	31.50	250				smooth								30 - 35						1 : 1.23	S	220				
MDDM	2M	2	500	19.69	250				smooth								30 - 35					X	1 : 1.23	S	220	7.5	10	0.313	0.025
MDDM	3M	2	500	19.69	250				smooth								30 - 35					X	1 : 1.23	S	220				
MDDM	QU	2	500	19.69	250				smooth								30 - 35					X	1 : 1.23	S	220	7.5	10	0.313	0.025
MDDM	4M	2	500	19.69	250				smooth								30 - 35					X	1 : 1.23	S	220				
MDDM	1T	2	500	19.69	250				smooth								30 - 35					X	1 : 1.23	S	220				
MDDM	5M	2	500	19.69	250				smooth								30 - 35					X	1 : 1.23	S	220	7.5	10	0.313	0.025
																									Total =			3.42	0.275
MDDM and MDDO: model # of roll stand (Buhler labeling)				Passage: BK=break; D= durum mill flow; SIZ= sizing; M= middling; QU= quality; T= tailing							Grinding: A = Sharp / Sharp D = Dull / Dull						Feed rolls:	W = Distribution screw S2 and PT5 = Types of feed rolls (Buhler labeling)						Scraper:	B= Brush S= Knife		Original from: Modifications:		JS JS 1/15/13	