Evaluation of extruded and germinated sorghum as a potential ingredient in dog and cat diets

by

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Abstract

Sorghum has many unique features that can be utilized in the pet food industry. However, its growth is hindered by outdated livestock literature and negative connotations. The aim of this thesis was to evaluate various sorghum accessions and preparations as an ingredient in extruded and raw pet foods for utilization by dogs and cats. The first study determined the processability of different sorghum pericarp varieties (white sorghum A, white sorghum B, burgundy sorghum, and sumac sorghum) compared to four small grains (rice, barley, oats, and millet). All four sorghum varieties produced well-expanded kibbles providing evidence for a good substitution for other small grains. Digestibility and oxygen radical antioxidant capacity (ORAC) of the sorghum containing diets were evaluated with 12 adult beagles in a 4 × 4 Latin square design experiment. Dietary treatments comprised 50% of white sorghum A or B, burgundy sorghum, or rice (as the control). No differences were observed in crude protein and fat apparent total tract digestibility ($P>0.05$). Furthermore, ORAC values were similar among all treatments ($P=0.4928$). Dogs fed the rice diet had higher plasma cholesterol levels compared to dogs fed the three sorghum treatments ($P=0.0007$); otherwise, diet had little influence on humoral constituents. This suggests that sorghum may be a good substitute for rice in canine diets.

Consumers are demanding “natural” and less processed – even raw diets for their pets. Raw diets consist of raw meat, bones, fruits, and vegetables. However, they cannot undergo any heating to be considered “raw.” Because carbohydrates typically require cooking, they are not commonly included in raw diets. Germination may solve this problem as it mobilizes the nutrients within the plant. To evaluate this, five common cereal grains (corn, millet, sorghum, soy, and wheat) were selected for evaluation. Seeds were germinated to initial sprout eruption and also allowed to grow into microgreens and then compared to the raw seeds. In general, a decrease in ash, fat (except for soy), resistant starch (except for soy), phytic acid (exception for corn and soy) and increases in *in vitro* protein digestibility (exception for wheat) were observed in the germinated seeds compared to their raw seeds. From this, sorghum was selected to evaluate further for seed growth, diet processing, and animal utilization.

Sprouts and microgreens were grown in a commercial microgreen tower (Throckmorton Hall, Kansas State University). Growth optimization was undertaken to determine the best substrate and seed density. Once a protocol was developed sprouts and microgreens were grown for food production. Raw diets containing 30% seeds were produced in the laboratory and then diets were pasteurized at high pressure (HPP) and verified for microbial safety at a commercial laboratory. Eleven shorthair cats were enrolled in a replicated incomplete 4 × 4 Latin square designed feeding study that consisted of 5 d adaptation followed by 5 d collection in each of the 4 periods. Treatments included raw sorghum, cooked sorghum, sprouts, and microgreens. Cats fed the sprouted and microgreen-based diets had similar dry matter, organic matter, and gross energy apparent total tract digestibility to the raw sorghum, and all were lower digestibility compared to the cats fed the cooked sorghum diet ($P < 0.05$). Among treatments, no differences were observed in protein digestibility ($P > 0.05$). However, fecal scores were the major difference in this study as cats fed the raw and sprouted diets had more diarrhea and loose stools compared to cats fed the microgreen and cooked diets. Further evaluation is needed on sorghum microgreens as an ingredient in raw diets. Overall, sorghum is a good substitute for other common grains as it can process well in an extruded diet, provides high levels of polyphenols, and provides potential for new products in raw diets.

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List of Abbreviations

SWOT- strengths, opportunities, weaknesses, and threats
BMI- body mass index
WSA- White sorghum A
WSB- White sorghum B
BS- Burgundy sorghum
SEI- sectional expansion index
TPC- total phenolic content
ME- metabolizable energy
ORAC- oxygen radical absorbance capacity
ATTD- apparent total tract digestibility
IR- intake ratio
DM- dry matter
OM- organic matter
CP- crude protein
TDF- total dietary fiber
IDF- insoluble dietary fiber
SDF- soluble dietary fiber
GE- gross energy
NFE- Nitrogen free extract
IVPD- *In vitro* protein digestibility

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Chapter 1 - Situational Analysis of Sorghum in North American Pet Foods

Abstract

Sorghum is a sustainable cereal which provides a well-rounded nutritional composition and contains polyphenolic antioxidants which may provide health benefits. However, its use in the pet food industry is limited. Therefore, the objective of this research was to conduct a systematic literature review regarding sorghum use in pet food to determine its benefits and limitations The review was overlaid on a common business tool the SWOT analysis (Strength, Weaknesses, Opportunities and Threats) to provide a framework to aid the evaluation. The literature review identified 39 applicable peer reviewed original research articles that were summarized. From 23 different sorghum-based diets the average protein digestibility was 82% when fed to healthy adult dogs. Average fecal scores and pH's were ideal. Sorghum showed potential to reduce glucose levels and body mass indices (BMI) while sorghum bran showed potential to increase antioxidant potential. Palatability was comparable to rice and wheat when combined with other ingredients. However, it was found that there is a deficiency of published research in the areas of palatability, antioxidants, glucose levels, gut health and weight loss regarding sorghum when fed to dogs or cats. More research is warranted in each of these areas to provide more information on the effects of sorghum on the overall health of companion animals.

Keywords: antioxidants, digestibility, glycemic, palatability, sorghum, SWOT

1. Introduction

In 2022, the value of the pet food industry was $50 billion, and it has continued to grow (Nielsen IQ, 2022). Additionally, COVID-19 led to 23 million American households acquiring new pets which increased the demand for pet food (*New ASPCA survey shows overwhelming majority of dogs and cats acquired during the pandemic are still in their homes*. (n.d.-b), Morrison, 2020). Dry cat and dog food are still the highest grossing type of food and highest tonnage produced (Decision Innovation Solutions, 2021). The largest ingredient class by proportion in dry pet food is starches, which are primarily from grains (or cereals). Approximately, 1.9 million tons of whole grain and 1.2 million tons of mill feed were used in pet foods (Decision Innovation Solutions, 2021). The top 5 grains used in pet food were corn, soybean, wheat, peas, and rice. In a report published by IFeeder/NARA (2021) top 13 plant-based ingredients for both dog and cat food were identified, and sorghum was conspicuously absent. This suggests that pet food as a trade is not on pace to use sorghum comparable to other animal industries. The question of course is why?

Perhaps it has to do with the way pets are viewed. Consumers think of their pets as family members rather than as mere animals in their home (Walsh, 2009). As a family member the decisions regarding nourishment and foodstuffs emphasize the human biases, sensory perspectives, and general preferences rather than considering the food as an economic input like the practice in animal agriculture. Consumers look at their pet’s food labels more than ever. This leads to an increasing demand for “natural” or higher quality and nutrient dense foods. Humanization of pet food led to consumers wanting more meat and less grain in their pets’ diets since they perceive grain to be lower quality. Grain-free diets became very popular during the 2000-2020’s for that reason. However, Nielson (2022) reports that since 2020 the “grain-free” fad has declined, and grain inclusive diets are making a comeback. Additionally, the industry has seen a rise in the use of high-quality sources of grains such as whole grains and ancient grains; the latter of which includes sorghum. This might provide an opportunity for an increased usage of sorghum. While not currently popular in pet diets it checks-off a number of criteria expressed as desirable by consumers. For example, sorghum contains high levels of polyphenols which have been reported to have anti-canter, anti-diabetic, anti-inflammatory, and cardiovascular health effects in humans, hamsters, mice and rats (Khoddami et al., 2021, Xiong et al., 2019, Xu et al., 2021). In fact, it is reported that sorghum has the highest antioxidant activity among cereal grains (wheat, rice, corn) and is comparable to fruits and vegetables (Xiong et al., 2019). Furthermore, Xiong et al. (2019) reported a brown sorghum bran extract had an *in vitro* oxygen radical absorbance capacity/antioxidant capacity (ORAC) of 3,124 µmol TE/g which is almost four times the ORAC of blueberries (ORAC= 842 µmol TE/g) which are known to be one of the higher antioxidant foods. Additionally, sorghum is of interest to those who have Celiac disease as it is gluten-free (Xu et al., 2021, Xiong et al., 2019, Khoddami et al., 2021, Zheng et al., 2023). Furthermore, sorghum has been used in human food for years to make tortillas, pasta, cookies, and porridges due to its rich source of vitamins and similar chemical and nutritional profiles to corn and wheat (Anglani, 1998, Khoddami et al., 2021). However, there are misconceptions that hinder the use of sorghum in pet foods. For example, protein digestibility is considered poor (Anglani et al, 1998). This is based on *in vitro* benchtop results (Duodu et al, 2003) or livestock feeding studies (McCollough and Brent, 1972, Streeter et al., 1990) but not for extruded dog/cat foods. Conversely, Etuk et al. (2012) and McCuistion et al. (2019) reported that feeding low-tannin sorghum varieties to swine did not affect feed conversion rates or efficiency. Furthermore, when processed correctly, sorghum has been reported to be able to fully substitute corn or wheat in poultry diets (McCuistion et al., 2019). However, for our companion animals there is generally a shortage of information regarding the use of sorghum in their foods - throughout retail outlets and with pet parents. The aim of our work was to determine what information is available and to summarize the findings to determine where gaps in our knowledge may exist. From this work one would provide the industry and supporting trade organizations with information that may help better identify the gaps that need to be addressed and better provide information to inform and educate the buying public with the ultimate aim to benefit the dog and cat.

2. Literature Review

2.1 Procedures for systematic search of the literature

To explore the topic in a systematic fashion, four reliable scientific databases were chosen to facilitate the search for relevant published research. The databases included Web of Science Core Collection, PubMed, CAB Direct, and Scopus. The Boolean search words chosen were "sorghum" "dogs" "canine" "cats" "felines" "feed" and "food." Combinations of these terms joined with "AND" were used to search for relevant articles. A total of 142 articles were found. After eliminating duplicate manuscripts from each database (n=71) there remained a total of 71 articles. Exclusion criteria included whether they evaluated sorghum in species other than dogs or cats (n=8), if they only included sorghum or just dogs or cats, if they did not use sorghum bicolor (n=7), or if they lacked relevancy (or data) to food, processing, or nutrition (n=17). After exclusion, there were 39 articles that were directly applicable which were then summarized. All articles were from research facilities and/or universities and several included information regarding chemical composition, digestibility, blood parameters, fecal parameters, glucose and insulin levels, processing, and palatability. All articles were read thoroughly, and common elements were summarized in tables (Tables 1-1 to 1-7) to provide a reader friendly approach to compare amongst research articles that addressed similar topics. Upon completion of the tabled summaries a thorough synthesis of the information followed.

3. Chemical composition of sorghum and sorghum co-products

Among the 39 articles sorghum (unspecified), white sorghum, red sorghum, whole sorghum, extruded sorghum, cooked sorghum, soaked sorghum, sorghum mill-feed and sorghum bran were considered. Sixteen of the 39 articles provided nutritional composition for the sorghum evaluated in the study. Two manuscripts evaluated sorghum flour (one specified it was red the other did not specify) and only one research study reporting results from sorghum mill feed and sorghum bran. Additionally, only two research papers reported data from extruded sorghums: one at a low temperature (84°C) and one at a high temperature (145°C). All remaining research manuscripts simply stated “sorghum,” “raw sorghum” and three specified the color of the raw sorghum.

Regarding nutrient composition, the dry matter composition ranged between 87.1 and 94.1% with a typical moisture of 10-12% amongst the various sources of the grain. The crude protein from the research summarized averaged 11.3% overall which is higher than is normally considered for sorghum - it is usually assumed to be approximately 9% on an as is basis. If we specifically consider the three red sorghum sources, the crude protein was 11.5% (excluding mill-feed). Further, if we exclude the sorghum mill feed and only consider the “grains,” the crude protein averaged 10.4%. For the extruded sorghum alone, the crude protein was 9.8%. Amongst the published research reviewed, crude fat ranged from 2.5-6% with an average of 4.04% for all ingredient categories and an average of 3.92% if sorghum mill-feed was excluded. Total dietary fiber ranged from 3.6 (flour) to 22.9% (sorghum bran) with an average of 11.95% with all ingredients included and 9.99% when sorghum bran and mill feed were excluded. The two sorghum flours averaged a total dietary fiber of 3.2%. Within total dietary fiber, insoluble dietary fiber averaged at 10.63% with all ingredients included and averaged 7.64% if sorghum mill feed and sorghum bran were excluded. Soluble dietary fiber averaged 2.75% with all ingredients included and 2.64% with sorghum mill feed and sorghum bran excluded. Crude fiber averaged 2.91% with only 4 papers reporting values. All papers reported ash at or less than 2.1%. Gross energy values averaged 4.57 kilocalories per gram with only 5 papers reporting values.

The starch composition of sorghum diets and ingredients from the four papers that reported this information on sorghum diets and 8 papers that reported information regarding starch analysis on sorghum ingredients are reported in Table 1-2. Diets had an average of 41.7% total starch with 73% of the starch was gelatinized. For sorghum ingredients the average was 72.2% for total starch. When mill feed was excluded the concentration of total starch was 73.5%. The rapidly digestible starch among ingredients averaged 44%, slowly digestible starch averaged 24%, and resistant starch averaged 9%. Starch gelatinization for those samples which were directly analyzed (i.e. not those done by Thivend method) averaged 76%.

4. In vivo digestibility of sorghum in diets in dogs (n=20) and cats (n=3)

There were 14 different articles with 23 different sorghum diets ranging from 16% inclusion to 99.85%. Twenty of the diets were evaluated by dogs and three for digestibility by cats. Dry matter digestibility ranged from 76% to 91% across all observations and organic matter digestibility ranged from 70 to 91% and averaged 84.5%. Fat digestibility ranged from 78% to 97% with an average of 89.1%. Crude protein digestibility ranged from 67 to 88%; the lowest value of which was the diet containing sorghum mill feed rather than sorghum grain. Total dietary fiber ranged from 27% to 56% digestible. Gross energy digestibility was 70 to 90%.

Starch was above high 90’s ranging from 94 to 100%. One common complaint about sorghum is the low protein digestibility, however, the average crude protein digestibility was 82%, where 80% digestibility or higher is considered acceptable. If only diets with 50% or more sorghum were considered, the average crude protein digestibility was 80.77% crude protein digestibility (with mill-feed included). If we exclude the mill feed and focus on sorghum grain and sorghum flours at 50% or greater inclusion, an average of 82.13% crude protein digestibility was observed. Therefore, sorghum used above 50% inclusion rate did not alter the crude protein digestibility negatively compared to the lower percentages found in these articles.

5. Fecal parameters and stool quality of dogs and cats fed sorghum diets

Eleven articles reported information regarding fecal parameters of dogs and cats fed 18 different sorghum diets. Three of the diets were fed to cats while the remaining 15 were fed to dogs. Not all fecal scores were reported in a similar manner across the various research institutions, so the scores were translated to a 1 to 5 scale where 1 as a watery diarrhea and 5 as a hard pellet and 3.5 to 4 is considered ideal score. Across all studies, the average score was 3.61, which is within the ideal range. If diets were above 50% inclusion rate the average fecal score was 3.55. This includes the coarse grind in the Bazolli et al. (2015) study. Most production facilities will not use a coarse grind although excluding this data point did not substantially change the fecal score average (3.65). The average fecal pH among reported studies was 6.28. Total short chain fatty acids concentration averaged 454.5 mmol/kg among the five papers that reported this information on it. Among the short chain fatty acids acetate averaged 250.6 mmol/kg of dry matter, propionate at 128.3 butyrate at 77.1 mmol/kg of dry matter and lactate was 14.1 µmol per gram.

6. In vitro fermentation of sorghum products

During the literature search, two studies were found that reported colonic fermentation information associated with sorghum. Murray et al. (1999) reported ileal fermentation by dogs and Donadelli et al. (2019) reported in vitro fermentation by dogs. Murray et al. (1999) published two experiments. The first looked at the difference between native, low temperature extrusion and high temperature extrusion sorghum compared to other grains with the same treatments and the second experiment evaluated the overall grains compared to each other. The sorghum treatments had higher organic matter disappearance when extruded at higher temperatures compared to lower temperatures. Ileal propionate values were significantly higher when diets were extruded at higher temperatures compared to lower temperatures. No differences were noted between high and lower temperatures for butyrate, lactate, acetate, or total short chain fatty acids. Sorghum was intermediate with corn and barley for native and low temperature sorghum butyrate production was only outperformed by rice. In experiment two, where just the raw grain flours were examined, the results were different. Murray et al. (2000) found that sorghum had an organic matter disappearance around 18% which was closest to rice at 23%. Sorghum was the lowest among the grains in acetate production and the results were most similar to rice. Propionate and butyrate were both relatively low as well and were similar to rice, wheat, and corn in these categories. Total short chain fatty acids were lower but still similar to rice. Overall, sorghum mirrored rice in organic acid production values. Sorghum was similar in most aspects to rice which is commonly used in the industry and known as a beneficial grain.

Donadelli et al (2019) evaluated different fiber sources and their impact on short chain and branched chain fatty acids in an in vitro fermentation model that relied upon dog feces as the inoculum. In this experiment they explored common fiber ingredients used in the pet food industry like beet pulp and pea fiber as controls versus more novel fiber ingredients like sorghum bran and Miscanthus grass. Donadelli et al. (2019) reported that sorghum bran produced the second highest levels of butyrate production (next to beet pulp) suggesting a benefit for gut health. However, the sorghum bran treatment also produced the highest levels of isovalerate, valerate, and overall branched chain fatty acids which have been reported to be produced by *Bacteroides* and *Clostridium* and can potentially harm the colon epithelium (Rios-Covian et al., 2020).

7. Glucose and insulin levels and opportunities for sorghum

Obesity and glucose insensitivity are prominent in pets (Association for Pet Obesity Prevention, 2022). In response, ingredients that can slow the release of glucose into the blood stream are being explored for pet food applications. Appleton et al. (2004) tested a rice-based diet compared to a corn and sorghum diet (33% starch where 50% corn and 50% sorghum) in overweight cats in two phases; a weight maintenance phase and a free-access phase and assessed weight gain when fed ad libitium. A sorghum/corn-based diet helped overweight cats with reduced insulin sensitivity, lowered insulin and glucose concentrations, caloric intake, and reduced weight gain compared to cats fed a rice diet. Carciofi et al. (2008) reported that sorghum was similar to peas and lentils for glycemic control in dogs, however, sorghum had superior crude protein and gross energy digestibility compared to peas and lentils. De Oliviera et al. (2008) repeated this study design in cats and reported that starch had less of an effect, however, corn did elicit a higher glucose response than sorghum. Teshima et al. (2021) found that feeding two equal meals and giving two equal insulin dosages resulted in a lower mean and minimum glycemia in the sorghum-based diet compared the rice diet. There was a trend towards a lower glucose area under the curve and maximum glycemia for the sorghum-based diet compared to the rice-based diet. However, when feeding three meals and giving two unequal insulin dosages no differences were observed. Teixeira et al. (2019) did not observe any differences between treatments of rice and sorghum with or without supplemental hydrolysable tannins on glycemia in adult dogs (2019). In a follow up study, Teixeira et al. (2021) also did not observe any differences between treatments with maize and sorghum with or without supplemental hydrolysable tannins in glycemia in dogs. Both studies may be explained by the different chemical composition between the sorghums used. Teixeira et al. (2021) reported that they used a high-tannin sorghum which has approximately 4.8% tannins - this may have caused the difference. More research may be needed to determine the differences among sorghum genotypes and how they affect the glycemic responses.

8. Antioxidant properties of sorghum

Another unique factor associated with sorghum are the polyphenolic compounds concentrated in the seed coat. These compounds have often been considered as antinutritional; however, they have recently been reconsidered as beneficial due to their rich concentration of polyphenolic antioxidants (Salim et al., 2023). Previously, in research publications focused on human nutrition and health sorghum has been reported to possess anti-carcinogenic effects and benefits for immune health (Cory et al., 2018). These polyphenolic antioxidants, if absorbed into the blood stream, might be able to reduce the harmful effects of free radicals and oxidized compounds in the body (Rana et al., 2022). Only one study has been published in pets regarding sorghum antioxidants. In this study, Alvarenga and Aldrich (2018) looked at the oxygen radical absorbance capacity (ORAC) in plasma of dogs fed three different sorghum containing diets. One diet contained whole red sorghum, another red sorghum flour, and a third red sorghum mill feed (bran). The dogs fed the sorghum mill feed containing diet had higher ORAC values than the dogs fed the whole sorghum diet or sorghum flour containing diets. The latter did not differ from the non-sorghum control. This may be a function of the polyphenols which are found in the pericarp, or outer layer, of the seed which is concentrated in the bran. That they were bioavailable in this form suggests that there is an opportunity to further enhance diets with these natural antioxidant compounds.

9. Blood serum levels of health markers in dogs and cats fed sorghum diets

Two studies evaluated complete blood counts and chemical profiles of serum in dogs. Feitosa et al. (2015) fed obese Dachshunds and Beagles with body conditions scores of 7 (1 to 9 scale) in the experiment. Dogs were weighed every two weeks and feeding amounts were adjusted accordingly. Fructosamine, total cholesterol, triglycerides, and glucose concentrations for dogs fed the corn diet had higher glucose values compared to dogs fed the sorghum and corn or sorghum only diets at 28 days. By days 56 and 112 there were interaction effects of breed and diet on blood glucose, total cholesterol, and triglycerides. Wherein, Dachshunds fed the sorghum diet had the lowest glycemic level compared to the other two diets. However, the Beagles had the highest cholesterol and triglyceride levels when fed the sorghum diets. On days 56-, 84-, and 112-days dogs fed with sorghum diet showed lower levels of fructosamine than dogs fed with corn. Teshima et al. (2021) did not see any differences in fructosamine, triglcerides, or cholesterol between a rice-based diet and a sorghum-based diet.

10. Weight loss in dogs fed sorghum diets

One study reported information on weight loss and BMI in dogs (Feitosa et al., 2015) as part of the experimental design when dogs were fed ad libitum for 60 days (weight gain phase) followed by assignment to one of three diets containing corn, a sorghum, or a mix of corn and sorghum. Dietary treatment did not affect overall body weight; however, there was a decrease in

BMI for dogs fed the sorghum containing diet. This suggests that sorghum may have influenced the glycemia and metabolic parameters associated with obesity in some manner. Though the mechanism is yet to be defined.

11. Palatability of sorghum-based pet foods

Four research publications provided some insight regarding the effect of sorghum on palatability or sensory properties. Addressing this aspect is meaningful since sorghum is often cited in human foods and livestock feed literature as being bitter or less accepted by animals from a palatability perspective. The published research pertaining to pets is quite different from that of other species. For example, Pezzali et al. (2021) reported that diets containing red sorghum and white sorghum versus rice crisps in cereal bars for dogs did not influence preference in dogs. Donfrancesco et al. (2018) examined the acceptability of dog foods made with whole red sorghum, red sorghum flour, and sorghum mill feed and found that pet owners had an impression of the shape, size and color. Most of the participants felt positive towards sorghum as an ingredient and those who did not primarily had the perception that it was a potential allergen (Donfrancesco et al., 2018). On a scale from 1 to 9 where one was dislike and nine was likes extremely, the whole sorghum diets scored higher than the other two diets in all categories (overall liking, appearance, and color) except aroma and the sorghum flour diet didn’t differ from whole sorghum in perceived color. Additionally, the whole sorghum diet resembled the control and was the closest cluster on the internal preference map which shows where similar sensory properties lie. While the whole sorghum diet ranked higher by pet owners it is important to mention that neither dogs nor dog owners rejected any of the diets. Lema Almeida et al. (2022) explored sensory perceptions of baked treats made with whole wheat flour, whole red sorghum flour, or whole white sorghum flour and it was found that they were comparable to each other. Further, ten dogs were used in a preference ranking test involving baked treats and did not show a preference (Lema Almeida et al., 2022).

12. SWOT Analysis of Sorghum in Pet Food

A SWOT analysis is a tool used to strategically plan and manage the organization (Gürel, 2017). The acronym SWOT stands for strengths, weaknesses, opportunities, and threats (sometimes exchanged for P for problems). By determining each of these areas in which sorghum exhibits an attribute business leaders and scientists can determine areas that need more attention, more education, or identify areas that have already been studied extensively and should be accentuated in communications to the public.

12.1 Strength of sorghum in pet food

Among Sorghum's strengths is its ability to tolerate harsh conditions like poor soil and droughts. With a predicted future population of 50 billion people this will become very important to feed a hungry world (Zarei, 2022). Additionally, sorghum has some highly marketable attributes for the health and wellness industry because it is gluten free (Khoddami et al., 2021, Xiong et al., 2019, Xu et al., 2021), contains polyphenolic compounds that can provide antioxidants (Khoddami et al., 2021, Xiong et al., 2019, Xu et al., 2021, Zheng et al., 2023) and is not genetically engineered (so-called non-genetically modified or GMO). In part because of its history of not being artificially altered genetically it has retained a perception as an "Ancient Grain" which provides a strong marketing position (McCuistion et al., 2019). Sorghum is also cost effective as a calorie source. According to Zarei et al. (2022), the price of sorghum was the same per pound as corn, and less expensive than wheat and soybeans. Cost containment is a very attractive quality to food production companies, especially when quality does not have to be compromised.

As shown in our summary, sorghum grain has a protein composition (10.4% crude protein) which is slightly higher than corn (7-9%; FeedTables, 2021). Among the 23 different diets that were evaluated in companion animals the digestibility was more than acceptable with crude protein digestibility averaging 82%. This clearly debunks the notion that sorghum is poorly digested. Among sorghum-based diets summarized, an average dry matter and organic matter digestibility of 82.9% and 84.5% were observed. Furthermore, an average fecal output of 43.5 grams per day (DM) were observed. Fecal output can be utilized as an indicator of digestibility (i.e. less feces means more digestibility and absorption of nutrients). From a consumer perspective this would best be viewed in terms of stool consistency; wherein, the average fecal score across all studies was 3.61 which places it within the ideal range for pet care and sanitation by the pet owner.

As a frame of reference, rice is a widely used grain in the pet food industry. Among the publications summarized, sorghum compared favorably to rice by in vitro digestibility. Rice is also known to be rapidly digested and to be high glycemic (Miller et al., 1992). Whereas a sorghum/corn-based diet was reported to lower insulin sensitivity, caloric intake, and reduce weight gain and performed more closely to legumes like lentils (Appleton et al., 2004, Carciofi et al., 2008). Thus, sorghum may be a valuable tool to aid in controlling glucose metabolism (Appleton et al., 2004, Carciofi et al., 2008). Further, body mass index (BMI) in dogs fed a sorghum diet were beneficially influenced without change to weight, which would suggest an improvement in retention of lean body mass at the expense of fat mass (Feitosa et al., 2015).

While all these attributes are valuable, probably the greatest benefit to sorghum beyond a mere carbohydrate and calorie source is the potent antioxidant polyphenolics contained in the seed coat (Khoddami et al., 2021, Xu et al., 2021, Xiong et al., 2019). As was noted by

Alvarenga and Aldrich (2018) sorghum bran increased circulating antioxidant capacity in dogs. The implications are that overall health and support to immune capacity may be improved with sorghum in the diet. The benefits of antioxidant capacity were noted without a decrease in palatability of the diet.

12.2 Weaknesses of sorghum in pet food

Probably the most significant weakness for sorghum as an ingredient in a pet food is the lack of name recognition or awareness about its nutritional contribution. Many consumers have not heard of sorghum and are not informed of its attributes (Ervina et al., 2023). For those consumers that have some recognition of sorghum it is typically regarding negative connotations of flavor (bitterness), poor digestibility (unfounded) and (or) as livestock feed or wild bird seed and not as a quality ingredient for pets (Xu et al., 2021, Zheng et al., 2023, Anglani, 1998).

Another shortcoming for companion animals is that many of sorghums attributes have been studied extensively in humans or with livestock. This gap makes direct translation difficult, especially given the differences in food/diet production and evaluation techniques amongst the species. Very few articles have evaluated the antioxidant potential, hematology, glycemia, weight management potential, or other potential health benefits of sorghum for dogs and even fewer in cats. For example, there is only one article published that explored the antioxidant capacity of sorghum in dogs or cats (Alvarenga and Aldrich, 2018). There was only one study that focused on weight loss for dogs fed sorghum diets even mentioned it (Feitosa et al., 2015, Appleton et al., 2004). There were few studies (6) that evaluated the influence of sorghum in the diet on glucose and insulin responses in dogs and cats. Without results to support claims for health benefits and(or) to debunk negative connotations sorghum will remain a minor ingredient in pet foods.

12.3 Opportunities for sorghum in pet food

There are many opportunities for sorghum in the pet food industry. For example, with a low glycemic index due to high resistant starch and total dietary fiber, sorghum may prove to be a valuable carbohydrate in diabetic diets (Khoddami et al., 2021, Xiong et al., 2019, Xu et al., 2021). Glycemia also has implications for obesity and weight control (Khoddami et al., 2021, Xu et al., 2021, Xiong et al., 2019). With over 59% of dogs and 61% of cats overweight or obese (APOP, 2022) control of weight and the underlying controls in this disease state could greatly benefit pets and their owners. Sorghum shows potential in glycemic control and caloric reduction and potential weight loss, but more data is needed to validate these results (Appleton et al., 2004).

The antioxidant aspects of the pericarp constituents from polyphenolics and other associated components may prove beneficial to animal health. Oxidative rancidity of foods is also an issue that shortens shelf life and reduces essential nutrients such as fat-soluble vitamins and essential fatty acids (Chanadang et al., 2016). Unleashing the antioxidants from the bran component might play a role in improving health, reducing waste, and improving the underpinnings of food sustainability (Gutierrez-del Rio et al., 2021). Previous work suggested there are opportunities for leveraging sorghum bran antioxidant potential (Alvarenga and Aldrich, 2018). It may also provide a valuable source of minimally fermentable bulking fiber for gut health (Donadelli et al., 2019). Most of the research in the pet arena regarding these topics has merely scratched the surface of the possibilities. Similar work has been published with applications in human foods and nutrition, some using surrogate species such as mice/rats (ref a couple). However, dog/cat food focused research is very limited with more questions than answers for their full application. Deeper and a wider breadth of work will be required before commercial pet food companies and pet food purchasers openly embrace these aspects of the ingredients in their products.

To that end, building awareness with consumers is the quintessential opportunity for sorghum in pet foods. Aspects of consumer interest are shifting towards whole grains and ancient grains- categories for which sorghum fits (Nielsen IQ, 2022). Ancient grains are perceived to be beneficial and higher quality than other grains (Nielsen IQ, 2022). Their status as non-GMO is also a positive attribute that should be evaluated for messaging that resonates with consumers. In these aspects of Sorghum original technical research is of limited value; whereas understanding likes and drivers for purchase by consumers could prove more beneficial.

12.4 Problems/threats for sorghum in pet food

The biggest problem or threat to sorghum is the negative perception it receives due to old literature. These negative connotations regarding low protein digestibility seem to linger. For example, Joye (2019) reports “sorghum proteins are poorly digestible, limiting the bioavailability of its amino acids” and “disulfide bonds between sorghum proteins during heat treatments leads to a lower protein digestibility.” However, there are many recent manuscripts that have evaluated sorghum and found diet digestibility similar to other grains in properly processed pet food applications. This sort of information needs to be repeated and promoted to break through the old myths. Additionally, sorghum has previously been reported to have a bitter taste. Educating consumers about their own diets and tastes will help with this effort. Because when used with other ingredients in a diet the prevailing pet research does not point to animal refusals. Case in point, neither Lema Almeida et al. (2022) nor Pezzali et al. (2021) observed differences between palatability results in which sorghum-based diets were compared to wheat based diets or when sorghum based diets were compared to rice based diets when fed to dogs. Again, better and more repeated communication is probably warranted if consumers' minds are to be changed to alter their perception about sorghum as an ingredient in pet diets.

13. Conclusions

Sorghum is a unique grain that is sustainable and requires less water than other cereals. It has a well-rounded nutritional composition at approximately 10% crude protein, 2-6% fat, and 3% fiber which compares favorably to popular grains in pet food such as corn and rice. It has often been saddled with the incorrect belief that protein digestibility is poor. Whereas actual feeding results prove otherwise – with average protein digestibility of sorghum grain and sorghum flour of 82%. When evaluating stool quality and health, across all studies summarized fecal scores and pH's were in the ideal range suggesting that animals fed sorghum-based diets were healthy. Additionally, sorghum reduced glucose peaks, lowered BMIs, and sorghum bran increased circulating antioxidant potential. The latter is merely a potential benefit that needs further investigation. More comprehensive assessments across all three domains (weaknesses, opportunities and threats) are required to thoroughly investigate the capabilities and constraints. Palatability has been evaluated and compared to rice in rice crisps, and to wheat flour in baked dog treats showing it can be a good substitute for other grains without issue. However, a big gap in our knowledge of consumer perception and reluctance to select sorghum as an ingredient remains. Sorghum has an opportunity to be utilized more in pet food if more detailed consumer information is linked to health benefits of sorghum in pet diets.

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Table 1-1 Proximate composition of sorghum ingredients from published research reported on a dry matter basis

Authors	Type	DM %	CP %	Fat %	TDF %	IDF %	SDF %	Crude Fiber %	Ash %	GE (kcal/g)
Carciofi et al. 2008	Sorghum	88.7	10.93	6.01	6.91	5.53	1.38	5.53	2.1	
Bazolli et al. 2015	Sorghum	88.8	10.25	3.83	12.61				1.2	
Fortes et al. 2010	Sorghum	89.0	11.22	3.15	10.79				1.02	5.06
Teixeira et al. 2019 and 2021	Sorghum	86.9	7.59	2.56	15.3			0.72	1.42	4.45
Von Schaumburg et al. 2021	Raw white sorghum	88.3	9.4	3.6	12.5	9.4	3.1		1.5	4.38
Von Schaumburg et al. 2021	Raw red sorghum	89.6	11.5	4.3	15.9	13.1	2.8		1.7	4.45
Murray et al. 1999	Sorghum flour	90.9	9.2	2.6	2.7				0.6	
De-Oliviera et al. 2008	sorghum	88.7	10.9	6.0	6.9				2.1	
Beloshapka et al. 2016	sorghum	88.6	10.4	4.3	12.5	9.9	2.7		1.7	4.5
Murray et al. 2000	Native sorghum	93.7	10.8	3.7					1.6	
Murray et al. 2000	Low temp extruded sorghum (84C)	94.1	10.2	3.4					1.6	
Murray et al. 2000	High temp extruded sorghum (145C)	93.6	9.4	4.0					1.4	

Alvarenga et al. 2018	Red Sorghum (whole sorghum)	87.06	12.06	3.23	10.11	7.12	2.99	1.36	1.38
Alvarenga et al. 2018	Red sorghum flour	87.63	11.05	4.20	3.65	0.80	2.85		1.19
Alvarenga et al. 2018	Red Sorghum Mill feed	88.51	15.14	5.67	22.60	20.68	1.81	4.02	2.04
Donadelli et al. 2019	Sorghum bran	93.9	20.8		22.9	18.5	4.4		

DM= dry matter, Ash=100-organic matter, CP= crude protein, GE= gross energy, TDF= total dietary fiber, IDF=insoluble dietary fiber, SDF= soluble dietary fiber

Table 1-2 Starch composition of sorghum diets and ingredients

Authors	Type	Inclusion, %	TS*	RDS	SDS	TDS	RS	SG %	DoC
Diets:									
Bazolli et al. 2015	Sorghum fine grind diet	53.4	41.4					86.7	
Bazolli et al. 2015	Sorghum medium grind diet	53.4	42.7					71.7	
Bazolli et al. 2015	Sorghum coarse grind diet	53.4	41.0					62.4	
Pezzali et al. 2019	Sorghum diet	16.78						32.96	86.65
Teixeira et al. 2019	Rice + sorghum	25						92.0	
Teixiera et al. 2019	Rice + sorghum + hydr tannins	25						92.3	
Ingredients:									
Murray et al. 1999	Sorghum cereal grain flour		89.7	63.5	24.6		1.6	89.7*	
Murray et al. 2001	Native sorghum		74.1	27.3	13.0		33.8	75.1	
Murray et al. 2001	Low temp extruded sorghum (84C)		75.4	49.1	10.9		15.4	75.4	
Murray et al. 2001	High temp extruded sorghum (145C)		77.5	70.0	5.4		2.1	77.5	
Lema Almeida et al. 2021	Whole red sorghum flours		78.9	29.3	41.2	78.34	0.56		
Lema Almeida et al. 2021	Whole white sorghum flour		81.4	24.5	46.7	80.98	0.47		
Alvarenga et al. 2018	Red sorghum (whole)		70.64						
Alvarenga et al. 2018	Red sorghum flour		76.46						
Alvarenga et al. 2018	Red sorghum mill feed		49.49						
Inal et al. 2017	Raw sorghum		74.4						

Inal et al. 2017	Grain/cereal	72.95
Inal et al. 2017	Soaked sorghum	73.11
Inal et al. 2017	Cooked sorghum 10 min	74.02
Inal et al. 2017	Cooked sorghum 20 min	73.84
Inal et al. 2017	Extruded sorghum	68.64
Teixeira et al. 2019 and 2021	Sorghum	63.6
Von Schaumburg et al. 2021	Raw white sorghum	66.4
Von Schaumburg et al. 2021	Raw red sorghum	59.2
De-Oliviera et al. 2008	sorghum	72.4

*Total starch (TS), Rapidly digested starch (RDS), Slowly digested starch (SDS), Resistant starch (RS), Starch gelatinization (SG), Degree of Cook (DoC).** by addition (84.8 by Thivend method)

Table 1-3 Essential amino acids, non-essential amino acids, and mineral composition (DMB %) of sorghum

Essential Amino Acids

Author	Type	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Total EAA
Beloshapka et al. 2016	sorghum	0.33	0.22	0.44	1.32	0.18	0.27	0.60	0.30	0.08	0.54	4.28

Non-Essential Amino Acids

Author	Type	Ala	Asp	Cys	Glu	Gly	Hyl	Hyp	Orn	Pro	Ser	Tau	Tyr	Total NEAA
Beloshapka et al 2016	sorghum	1.12	0.62	0.15	2.23	0.28	0.01	0.01	0	0.73	0.58	0	0.26	5.99

Minerals

Author	Type	Ca	Cl	Mg	P	Phytic Acid	K	Na	S
Beloshapka et al. 2016	sorghum	0.02	<0.10	0.18	0.36		0.40	<0.10	0.10
Fortes et al. 2010	sorghum	0.02			0.12				
Kore et al. 2009	sorghum	0.64			0.53				
Von Schaumburg et al. 2021	Raw white sorghum					1.1			
Von Schaumburg et al. 2021	Raw red sorghum					1.1			

Table 1-4 In vivo digestibility of dogs and cats fed sorghum-containing diets

Author	Type	Inclusion %	Species	DM	OM	CP	Starch	Fat	GE	Crude fiber	TDF	NFE
Pezzali et al. 2020	Grain based	16.86	dog		87.27	86.86		90.59				
Pezzali et al. 2019	sorghum	16.78	dog	85.8	87.7	88.1	99.6	93.1	87.5		39.3	
De Silva Junior et al. 2005	Sorghum diet	21	dog	82.68			95.10					
Teixeira et al. 2019	Rice + sorghum	25	dog	78.5	81.9	82.3		89.4		30.8		
Teixeira et al. 2019	Rice + sorghum + hydr tannins	25	dog	76.7	80.9	79.6		90.3		33.7		
Teixeira et al. 2021	Maize and sorghum and hydrolysable tannins	26.2	dog	78.2	81.7	79.5		83.2	81.3			82.1
Texiera et al. 2021	Maize and sorghum	26.7	dog	80.6	83.4	78.4		86.2	83.0			84.2
Fortes et al. 2010	sorghum	30	dog	86.6	88.5	85.0	98.9	89.5		33.1		
Fortes et al. 2010	Sorghum extruded and calc by differential method	30	dog	90.5	89.6	88.4	98.7	78.6		74.1		
Von Schauburg et al. 2021	Raw white sorghum	30	cat	81.0	86.4	86.0		92.8			56.0	

Von Schaumburg et al. 2021	Raw red sorghum	30	cat	81.1	86.4	86.6		92.8			53.2
Bazolli et al. 2015	Fine grind	53.4	dog	83.2		83.6	99.7	94.3	86.7		
Bazolli et al. 2015	Medium grind	53.4	dog	79.9		80.1	99.1	92.8	84.4		
Bazolli et al. 2015	Coarse grind	53.4	dog	75.9		74.9	97.8	91.4	80.2		
Twomey et al. 2003	Sorghum diet	55.2	dog	94		85	100	96	87		
Twomey et al. 2003	Sorghum + enzyme diet	55.2	dog	94		85	100	97	87		
De Oliviera et al. 2008	Sorghum diet	57.42	cat	76.3	80.0	80.6	93.9	83.3	79.6		29.0
Carciofi et al. 2008	Sorghum diet	59.27	dog	79.0	83.8	85.0	99.18	88.3	84.2	3.7	27
De Silva Junior et al. 2005	Sorghum diet	63	dog	81.11			94.68				
Alvarenga et al. 2018	Sorghum flour	63.11	dog	86.0	90.7	81.8		91.4	90.3		30.2
Alvarenga et al. 2018	Sorghum mill-feed	67.6	dog	65.9	70.6	67.2		77.9	70.2		10.3
Kore et al. 2009	sorghum	70.5	dog	83.1	85.1	94.3		85.9			49.6
Duarte et al. 2006	Sorghum	99.85	dog	86.43	87.48	71.01		86.88	87.23	0.09	

DM= dry matter, OM=organic matter, CP= crude protein, GE= gross energy, TDF= total dietary fiber, NFE= nitrogen free extract

Table 1-5 Fecal parameters of dogs and cats fed sorghum-containing diets

Author	Type	Inclusion %	Species	Fecal Score	Fecal DM	Fecal pH	Output (g/dog/day DM)	Acetate mmol/ kg DM	Butyrate mmol/kg DM	Propionate mmol/kg DM	SCFA (mmol/kg)	Lactate (umol/g)
Pezzali et al. 2019	Sorghum	16.78	dog	3.15	33.9							
Teixeira et al. 2019	Rice + sorghum	25	dog	3.87	38.4	6.60	43.2					
Teixeira et al. 2019	Rice/sorghum + hydrolysable tannins	25	dog	3.89	37.6	6.65	46.2					
Texiera et al. 2021	Maize/sorghum +Hydrolysable tannins	26.20	dog	3.99	35.6	6.54	46.7					
Texiera et al. 2021	Maize and sorghum	26.7	dog	3.88	34.3	6.44	40.2					
Von Scahumburg et al. 2021	Raw white sorghum	30	cat	3.50		6.3	14	231.6	44.8	82.9	359.3	
Von Schaumburg et al. 2021	Raw red sorghum	30	cat	3.70		6.3	14	263.4	45.8	94.8	404	

Bazolli et al. 2015	Fine grind	53.4	dog	3.3	38.9	6.7		305	88	212	605	11.9
Bazolli et al. 2015	Medium grind	53.4	dog	3.3	36.2	5.9		214	53	133	390	16.1
Bazolli et al. 2015	Coarse grind	53.4	dog	2.6	35.8	6.0		239	154	119	514	14.2
Fortes et al. 2010							63.5					
Twomey et al. 2003	Sorghum diet	55.2	dog	3.95	31.5	5.89						18.3
Twomey et al. 2003	Sorghum + enzyme diet	55.2	dog	3.52	33.3	5.85						13.1
De Oliviera et al. 2008	Sorghum diet	57.42	cat	3.2	31.5	5.7	17.3					
Carciofi et al. 2008	Sorghum diet	59.27		3.8	40.3	6.7						
Alvarenga et al. 2018	Sorghum flour	63.11	dog	3.78			32.6					
Alvaranga et al. 2018	Whole sorghum	64.7	dog	3.68			55.7					
Alvarenga et al. 2018	Sorghum mill feed	67.6	dog	3.92			95.4					

Kore et al. 2009	Sorghum diet	70.5	dog	4.0	16.9	6.3	20.0								11.2

Table 1-6 Dog and cat glycemic response to diets containing sorghum

Authors	Type	Inclusion, %	Species	Time to peak (min)	AUC 0-480 min	Basal G	Max G	Mean G	Min G	Max G increase
Carciofi et al. 2008	Sorghum diet	59.27	dog	160				80.5		
Teixeira et al. 2019	Sorghum + rice	25	dog		40350	78.2	99.7	83.1	68.7	21
Teixeira et al. 2019	Sorghum + rice + hydrolysable tannins	25	dog		39615	80.0	97.2	82.2	71.2	17.2
De Oliviera et al. 2008	Sorghum diet	57.42	cat	384	792.2		73.4	65.5		
Teixeira et al. 2021	Maize and Sorghum	26.7	dog		38633	80.3	97.0	80.2	66.7	16.7
Teixeira et al. 2021	Maize +sorghum+ hydrolysable tannins	26.2	dog		38884	81.3	96.0	82.0	69.5	14.7
Teshima et al. 2021	Sorghum Exp 1	42	dog		2180		244.0	160.2	90.9	
Teshima et al. 2021	Sorghum Exp 2	42	dog		1327.9		315	178.1	81.3	
Silva Junior et al. 2005	Sorghum diet	63	dog					52.08		
De Silva Junior et al. 2005	Sorghum diet	21	dog					55.20		

*Glycemia (G)

Table 1-7 Dog and cat insulinemic response to diets containing sorghum

Authors	Type	Inclusion	Species	Mean conc I	Mean I conc	Max I conc	Peak I conc	Time to peak (min)	AUC 0-300 min	AUC 0-30 min	AUC 30-300 min
Carciofi et al. 2008	Sorghum diet	59.27	dog	20.3	15.5		30.7	105.0	7749	491	7271
De Oliviera et al. 2008	Sorghum diet	57.42	cat	19.7	18.0	28.2		270			
De Silva Junior et al. 2005	Sorghum diet	63	dog	15.95							
De Silva Junior et al. 2005	Sorghum diet	21	dog	15.29							

*Insulin (I)

Chapter 2 - The comparison of sorghum cultivars to other common small grains used in in pet food formulations for their impact on processing, nutritional composition, and phenolic acid content

Abstract

In comparison to other small grains used in pet food applications sorghum provides a unique opportunity to fortify pet food diets with antioxidants in a sustainable way. However, the current utilization of sorghum in dog and cat food is low. This may be due to a lack of understanding by producers regarding its impact on processing and its chemical changes that follow. Therefore, the objectives of this study were to determine the effects of exchanging various small grains, grains that produce small seeds (wheat, barley, oats), in a dog diet on processing, finished product output, and the nutritional composition, including phenolic acids. Diets mimicking a standard dog food were produced containing 50% of grains from rice, barley, oats, millet or four cultivars of sorghum using a single screw extruder. Samples from each diet were sampled off the extruder and dryer at three different time points, twenty minutes apart for proximate analysis and kibble characteristics. The oat-based diet had the highest level of crude protein and fat while the millet-based diet had the highest fiber primarily as insoluble dietary fiber ($P<0.0001$). The rice diet had the lowest bulk density and highest sectional expansion index while the oat diet had the highest bulk density and lowest sectional expansion index ($P<0.0001$). The barley diet resulted in the hardest and toughest kibbles while the sumac and millet diets resulted in the least. Diets incorporating millet, sumac, white sorghum A and B, burgundy sorghum, barley, and rice yielded well-expanded kibbles whereas oats may require further refinement to processing parameters to achieve comparable finished product characteristics. Considering their textural attributes, white sorghum A and B, along with burgundy sorghum were the most suitable sorghum cultivars in this study for substituting other grains in the pet food industry.

Key Words: Sorghum, Pericarp, antioxidant, color, phenolics, grains

1. Introduction

Sorghum, a crop that is native to Africa, has the potential to be utilized for food processing through its functional starches and antioxidant phenolics (Alvarenga and Aldrich, 2018). In terms of functionality, the phenolic compounds present in sorghum may provide health benefits (Girard and Awika, 2018). These compounds consist of small molecules derived from cinnamic or benzoic acid (Ratnavathi, 2019). Phenolic compounds can stabilize free radicals naturally produced by the body during metabolic reactions, which if left unaffected, can build up and damage cells (Davis et al, 2019). Therefore, consumption of food containing high levels of phenolic compounds has become increasingly popular in the human industry due to their potential antioxidant properties (de Araújo, 2021) and gut health benefits (Girard and Awika, 2018). Some sorghum varieties are known to contain high levels of polyphenolic compounds (Girard and Awika, 2018), with darker color varieties usually presenting higher concentrations (Davis et al, 2019). These compounds are also usually found in higher concentrations in the outer layer of the sorghum grain, with the bran containing the highest levels. Sumac sorghum, for example, has the highest total volume of antioxidants compared to other sorghum varieties (e.g., white sorghum; Awika et al., 2009). The health benefits of sorghum have also been explored in dogs. Alvarenga and Aldrich (2018) reported an increase in fasted antioxidant capacity of healthy adult dogs fed a sorghum mill feed-based (67.6 % as-fed basis) extruded diet. However, the authors did not report the total phenolic content of the sorghum fractions used in the diets. Obtaining a comprehensive understanding of the total phenolic compounds in sorghum compared to other grains would enhance our knowledge of how substituting sorghum for common grains can influence the overall phenolic content of the diet and, consequently, the potential health benefits for the animal.

According to the IFeeder/NARA pet food report, sorghum was not within the top thirteen plant-based ingredients used in dog or cat diets (Decision Innovation Solutions, 2021). Corn, wheat, and rice (all of which did make the top 13 ingredients; Decision Innovations Solutions, 2021) as well as barley, oats, and rye (Lambrakis and Kersey, 2021), are examples of more commonly utilized grains in the pet food industry. Consumers tend to dislike small kibbles; thus, a well-expanded product plays an important role in consumer acceptance (Di Donfrancesco et al., 2014, Gomez Baquero et al., 2018). Additionally, product expansion and texture are important attributes that influence the dog’s feeding experience. While there are few studies that investigated the impact of different sorghum fractions and inclusion rates on extrusion of pet food (Alvarenga et al., 2018, Bazolli et al., 2015, Pezzali and Aldrich, 2019), there is still a lack of published data comparing how different sorghum varieties behave during extrusion and how they compare to other commonly utilized grains. Evaluating the behavior during extrusion and the content of phenolic compounds of different sorghum grain varieties compared to other grains can provide valuable data to the industry and increase awareness of the use of sorghum in pet diets. We hypothesized that both sumac and burgundy sorghum would have higher concentration of phenolic compounds compared to other grains and that all grains would behave similarly during the extrusion process, yielding kibbles with similar characteristics. Therefore, the objectives of this study were to 1) determine the nutritional and phenolic composition of different sorghum varieties (white sorghum, burgundy and sumac) and rice, oat, millet, and barley; and 2) evaluate the effect of these different grains on the extrusion process and kibble characteristics.

2. Materials and Methods

2.1 Ingredients and dietary treatments

Eight nutritionally complete and balanced diets were formulated to meet or exceed the recommendations for adult dogs at maintenance set by the Association of American Feed Control Officials (AAFCO, 2023). All diets were formulated to include 50% of the formula from each of the respective experimental grains. Two white sorghum varieties (WS A and WS B), burgundy sorghum (BS), and sumac sorghum (NuLife Market, Scott City, KS), millet, barley, oats, and rice (Anchor Ingredients, Fargo, ND) were sourced from leading ingredient suppliers. The remaining 50% of the diet was composed of a common basal mix sourced from a local mill (Fairview Mills, Seneca, KS), with chicken fat and dog digest applied topically after extrusion production of the kibbles. The chicken fat was supplied by a renderer (International Dehydrated Foods; Monett, MO) and the canine digest flavor by a pet food flavoring company (AFB International; St. Charles, MO). Titanium dioxide was added at 0.4% as an external marker for the subsequent feeding study.

2.2 Diet production

The diets were extruded on two separate days at the extrusion processing laboratory on campus (Bioprocessing & Industrial Value-Added Products Innovation Center at Kansas State University, Manhattan, KS) using a single screw extruder (Wenger Manufacturing Inc, Sabetha, KS). The screw profile included an inlet screw, single flight full-pitch screw, small shear lock, double flight half pitch screw, medium shear lock, large shear lock, and a double flight half-pitch uncut cone screw. At the diet plate kibble exited through a single 6 mm diameter diet and kibbles were cut with a 6-knife high speed rotating blade. After extrusion, kibbles were conveyed to a horizontal dual pass dryer and single pass cooler (Model 4800; Wenger Manufacturing Inc, Sabetha, KS). The dryer was set at 107°C and kibbles spent 6 min per pass (12 min total) and 5 min in the cooler. Samples out of the extruder and out of the dryer were collected at three time points, twenty min apart, following processing stabilization. Extrusion processing inputs were held constant among all diets to determine the impact each grain had on product quality. However, exceptions were permitted in order to produce a suitable kibble for feeding and sensory evaluation. Bulk density of the product out of the extruder and drier were measured. using a 1 L cup and a scale with 0.01 g precision.

2.3 Extrudate characteristics

Diameter and length of 10 randomly selected kibbles out of the dryer per time point of each diet were measured with digital calipers. Mass of the same kibbles were recorded with 0.0001 g precision. Length, diameter, and mass were used to determine the sectional expansion index (SEI), specific length (ls) and piece density.

Sectional expansion index was calculated as follows:

$$SEI = \frac{D^2}{d^2}$$

Where D is the extruded kibble diameter (mm) and d is the extruder die diameter (mm).

Specific length was determined by the equation below:

$$Specific\ Length\ (\frac{mm}{g}) = \frac{l}{m}$$

Where l is the extruded kibble length (mm) and m is the extruded kibble’s mass (g).

Piece density was calculated as follows:

$$p = \frac{m\ (g)}{V\ (cm^3)}$$

Where m is the kibble's mass (g) and V is the kibble's volume (cm^3).

Texture analysis was performed using a TA-XT2 Texture Analyzer (Texture Technology Corp., Scarsdale, NJ, USA) with a 30 kg load cell. A 25 mm diameter cylindrical probe was used to compress 30 kibbles from each diet. A pre-test speed of 2mm/s, test speed of 1mm/sec, and post speed of 10mm/s were used according to Dogan and Kokini (2007). Kibble hardness was the peak force of the first major breakage (N), and toughness was calculated as the area under the curve (N*mm).

2.4 Extraction of phenolics from raw ingredients and phenolic acid composition.

Phenolic compounds in the raw grains were extracted according to Lu et al. (2014) with modifications. Briefly, from each grain two grams of flour were extracted with 20 ml of 2M NaOH in 20% ethanol mixture in a 50 ml centrifuge tube. The mixture was left to shake in the dark for 3 h. Then, using concentrated HCl the pH was adjusted to 2 and the mixture was centrifuged at 10,000 g, at 20 °C for 15 min. The supernatant from each tube was collected and transferred to a new tube. The remaining mixture was extracted with equal amounts of ethyl ester and left to shake in the dark for 30 min. The tubes were then centrifuged (same conditions) and the supernatant was collected again. This step was repeated three times. The combined supernatant was evaporated using a nitrogen evaporator and stored at -20 °C until analysis.

The organic phase from the extraction was reconstituted with 1 ml of high-performance liquid chromatography grade methanol in a 5 ml centrifuge tube. Ten microliters of each sample were injected into an ultra-performance liquid chromatography for phenolic acid analysis.

2.5 Total phenolic content analysis

The total phenolic content (TPC) assay was performed according to Tian and Li (2018) with modifications. The organic phase from the extraction was reconstituted with 3 ml of high-performance liquid chromatography grade methanol in a 5 ml centrifuge tube. Digested supernatant (0.1 ml) was thoroughly mixed methanol (7.9 ml) and Folin-Ciocalteu reagent (0.5 ml). After 10 min, the mixture was added to Na_2Co_3 solution (20%, w/v). After resting in the dark for 2 h, the absorbance of the final mixture at 765 was recorded using a spectrophotometric plate reader (Synergy H1, BioTekInstruments, Inc.,Winooski, VT.). Samples were analyzed using gallic acid as an external standard and expressed as microgram of gallic acid equivalence (GAE) per gram of the original sample (µg GAE/ml).

2.6 Nutrient analysis

Raw grains and diet samples (coated and uncoated) were analyzed for moisture (AOAC 930.15), ash (AOAC 942.05), fat by acid hydrolysis (ISO 11085:2008), crude protein as nitrogen (AOAC 990.03) using a nitrogen analyzer (FP928, LECO Corporation, Saint Joseph, MI), gross energy (Parr 6200 Calorimeter, Parr Instrument Company, Moline, IL) and total dietary fiber (AOAC 991.43). Uncoated samples were analyzed in triplicates at the aforementioned time points during processing.

2.7 Statistical analysis

Extrusion data and nutrient composition from the uncoated diet were analyzed as a completely randomized design using the GLM procedure in statistical software (SAS v. 9.4, SAS Institute, Inc, Cary, NC). Diet was treated as a fixed effect and means were separated using the Tukey-Kramer adjustment. Differences among means were deemed significant at $P < 0.05$.

3. Results

3.1 Nutrient analysis of raw ingredients

Overall, the nutritional composition of rice differed the most from the other raw grains analyzed (Table 2-1). Dry matter (DM) ranged from 88 to 90% and ash ranged from 0.6% to 4.3%, with rice and millet containing the greatest and lowest ash concentration, respectively with the other grains containing an average of 1.6% ash (DM). Crude protein was the lowest in rice (9.5%) and the greatest in oats (14.5 %), with the other grains containing approximately 10-12 % (DM). Rice and barley contained the lowest crude fat concentration (1-2.5%) and oats contained the greatest (6.5 %), with other grains containing between 3-5%. The lowest total dietary fiber content was observed in rice at 3.8%, while WSA, WSB, BS, and millet exhibited the highest levels ranging from 22% to 28%. Barley, sumac, and oats contained 14-19% total dietary fiber, with the predominant composition being insoluble fiber. Except in the cases of barley and oats, over 85% of the total dietary fiber consisted of insoluble fiber. Soluble fiber was greatest for barley and oats at 3.6 and 4.4 %, respectively, whereas all other grains were less than 1 %.

Total phenolic content measures the total level of phenolics present in the grain. Sumac contained the greatest total phenolic content of all the raw ingredients (>3100 µg/g), rice contained the lowest levels at less than half the total phenolic content compared to all the other grains (<300 µg/g; Table 2-2). The seven phenolic acids analyzed were gallic acid, 4-hydroxybenzoic acid, syringic acid, sinapic acid, vanillic acid, ferulic acid, and p-coumaric acid. Sumac had the greatest levels of gallic acid and 4-hydroxybenzoic acid. Millet contained the greatest levels of vanillic acid, ferulic acid, and p-coumaric acid. Oats had the greatest levels of syringic acid and sinapic acid. Rice had the lowest levels of 4- hydroxybenzoic acid, vanillic acid, p-coumaric, ferulic acid and total phenolic content (<300 µg/g). WSA and B and BS had the lowest levels of sinapic acid, but intermediate levels of the remaining acids and total phenolic content (>600 µg/g).

3.2 Extrusion Processing

Feed rates of the raw materials did not differ (P > 0.05) among treatments. Discharge temperatures from the preconditioner were the greatest in the WSB diet (102 °C) compared to the rice diet (100 °C; P=0.0294). No differences were observed in steam injection in the preconditioner among diets (P>0.05). Water injection in the preconditioner was greater (P<0.05) in WSA, BS, and rice diets (10.97 kg/h) and lowest in WSB, millet, sumac, and barley diets (9.67, 9.70, 9.77, and 9.97 kg/h). Nevertheless, in light of the considerable distinctions observed in preconditioner discharge temperature and the introduction of steam and water injection, it is noteworthy that the numerical differentials did not attain a magnitude significant enough to have practical implications. Water injection in the extruder differed among diets. The oats containing diet required nearly one and half to two times the amount of water injection into the extruder compared to the other diets (20 kg/h vs. 10-14 kg/h; P<0.0001). The extruder speed was higher (P=0.0010) in the oats containing diet (482 rpm) and lowest in the millet, barley, sumac, and WSB diets (380 rpm for millet, barley, and sumac and 387 rpm for WSB). The knife speed was also higher (P<0.0001) in the oats containing diet (2535 rpm) compared to the other seven diets (2046 rpm). Motor load was greatest in the rice diet (57.3) and lowest in the oats and sumac diets (42.7 and 49.3; P=0.0001). Die pressure was higher (P=0.0081) in the barley diet (300 psi) and lowest in sumac, millet, and WSB diets (200 and 217 psi). Die temperature off the extruder was greatest in the WSB and barley diets (107 and 109 °C) followed by the oats, millet, sumac, BS, and rice (98-102 °C) diets. BS and rice were similar to WSA diet, which had the lowest diet temperature off the extruder (95.67°C; P<0.0001).

3.3 Nutrient Analysis of Diets

Diet samples taken off the extruder in triplicate samples were used for nutrient analysis. The barley diet had the greatest DM at 82 %, whereas oats and sumac diets had the least dry matter at 74 and 79 %, respectively (Table 2-4; $P<0.0001$). Organic matter did not differ among diets. Differences were noted in crude protein, fat, and gross energy, however, similar to some extrusion parameters, these variations were not substantial enough to have practical applications (i.e. 3-4% numerical difference in fat and protein percentages; DM). Conversely, the millet diet exhibited a significantly higher total dietary fiber content (24.5 %; DM) compared to the other seven diets containing 10-16%, primarily driven by its elevated levels of insoluble fiber. Soluble digestible fiber was significantly greater in the oat diet containing 5.7 % compared to the rice, WS A and B diets containing only 2.6-3.1 % (DM).

3.4 Kibble Traits

The oats diet exhibited greater wet and dry bulk densities (514 and 489 g/L, respectively; $P<0.0001$), while the rice diet displayed a lower wet and dry bulk densities compared to all other diets (316 and 305 g/L, respectively; $P<0.0001$). The remaining diets displayed intermediate characteristics and exhibited similar bulk densities. While these differences were statistically different, they are not substantial enough for practical application (<40 g/L differences). The rice and BS diets were the greatest piece volumes (0.57 cm^3) compared to the oats containing diet, which in turn had the lowest (0.24 cm^3 $P<0.0001$) among the treatments. The oats containing diet had a greater ($P<0.0001$) piece density (0.73 g/cm^3) compared to the other seven treatments with densities that ranged from 0.40-0.48 g/cm^3. The sectional expansion index (SEI) was greatest for the rice containing kibbles (3.65), followed by WSA, WSB, BS, and barley containing kibbles (2.8-3.0) which were higher than sumac and millet kibbles (2.33-2.36), with the oats containing kibbles exhibiting the lowest SEI (1.75; P<0.0001). Specific length was greatest in the barley diet (5.36) and lowest in the BS diet (3.80; P<0.0001). The kibbles produced with barley were harder and tougher (6.68 kg and 57.72 kg mm, respectively) compared to the others produced with different grains, whereas kibbles produced with millet and sumac required the least force to break (2.04 kg and 16.0 kg mm, respectively).

4. Discussion

One of the goals of this study was to compare the composition of several small grains used in pet food and how they impacted a formula as the sole source of starch (50% of each experimental diet). The nutritional composition of the grains analyzed in this study were relatively similar, with the exception of total dietary fiber. All grains expect rice exhibited high levels of total dietary fiber, which was mostly driven by the high insoluble dietary fiber content. On the other hand, the phenolic composition was substantially different among cereal grains. Rao et al., (2018a) reported three red sorghum varieties containing TPCs ranging from 660-880 µg/g and a white sorghum variety containing 240 µg/g. Compared to this study, their burgundy sorghum varieties had slightly lower values and their white sorghum variety had almost half the phenolic levels that were present in this current study. Min et al., (2012) reported that white rice grown in the United States contained 440 µg/g of total phenolic content, which was almost twice the amount that was reported in this study. Unpigmented barley was reported to contain approximately 1200 ug/g, and whole grain oat flour was observed to have a range containing from 200-1560 µg/g (Rao et al., 2018b). The discrepancy among studies is most likely due to the differences in temperature, salinity of soil, and water deficiency (Rao et al., 2018b). However, each grain had a unique phenolic acid composition, which typically aligned with literature (i.e. rice's highest phenolic acid was ferulic acid; Rao et al., 2018b). It is important to note that only one batch of each ingredient was sourced, preventing us from running statistical analysis due to the lack of replicates. Phenolic acids present in sorghum, such as flavones, tannins, and 3-deoxyanthocyanins, have been assessed for their ani-inflammatory properties in mice and rats (Girard and Awika, 2018). For example, extracts of sorghum phenolic reduced crypt foci formations in the colon of ovariectomized mice (Yang et al., 2014) and decreased fat accumulation and inflammation markers were observed in obese rats fed extruded sorghum flour diets (Arbex et al., 2018). Considering that consumers are looking for diets that promote the health and well-being of their pets, including ingredients with higher levels of phenolic compounds may be of benefit. Rice, which is one of the most utilized grains in the pet food industry, has a much lower concentration of phenolic compounds. Replacing rice with other grains that contain higher amounts of phenolic compounds, such as burgundy sorghum, sumac, or millet, may provide functional attributes to the diet. However, although the inclusion of grains high in phenolic compounds in pet food may be beneficial for pet health, it is important to understand whether they can be included in extruded pet food without altering the quality of the kibble.

Extrusion is dependent on the application of mechanical and thermal energy to facilitate the cooking and gelatinization of starch, induce expansion, and shape the final product (Baller et al., 2021). All four sorghum cultivars extruded well and produced well-expanded kibbles. Although they had a lower SEI and specific length than rice, the piece densities were similar. The SEI's for both white sorghum varieties and burgundy sorghum were high relative to the remaining grains. The process of expansion plays a crucial role in determining the texture and palatability of the final product (Koppel et al., 2015; Souza et al., 2022). Oats contained greater levels of total phenolic compounds compared to rice, WSA and WSB, however, the inclusion of oats at 50% compromised kibble expansion. The higher kibble density observed in the diets produced with oats corroborates with previous studies in which adding oat flour (Holgui-Acuna et al., 2008 up to 50% inclusion rate, Liu et al., 2000 at 55-100%, and Sandrin et al., 2017 at 60%) to an extruded breakfast cereal or snack formula reduced the expansion of the extrudates. The reduction in expansion for oats may be explained by the greater fat content of oats compared to the other cereal grains evaluated, which resulted in a higher internal fat addition in the process. Fat acts as a lubricant during the extrusion process, decreasing the friction between the material and the extruder barrel, and consequently, the mechanical energy input (Nikinmaa et al, 2023, Lin et al, 1997, Rokey et al, 2010). The oats containing diet also required greater addition of water in the extruder, which also adds in lubrication and reduces friction (Lin et al, 1997, Rokey et al, 2010, Alam, 2012). The lower the mechanical energy input, the lower the product expansion (Agbisit et al., 2007, Karkle et al., 2012, Alam, 2012). Unfortunately, we were not able to calculate specific mechanical energy due to the lack of motor power data in all dietary treatments. However, the lower kibble expansion in the oats diet compared to the others is likely due to a lower input of specific mechanical energy as a result of a higher internal fat and water addition during extrusion. A greater increase in the screw speed, which inputs more heat and energy, may be necessary to produce less dense kibbles in extruded pet food containing high levels of oats.

Product texture is also important to evaluate as texture influences the mouthfeel and palatability of a product (Baller et al., 2021, Koppel et al., 2015). Kibble hardness, defined as the material resistance to deformation, is an important property to evaluate product texture. A greater energy input usually leads more expanded and less hard kibbles (Baller et al., 2021, Pacheco et al., 2018). In our study, however, the kibbles produced with oats had lower hardness compared to those produced with rice, even though the former was less expanded. The same trend was observed for kibble toughness between the diets produced with rice and oats. The likely higher energy input in the rice diet could have led to the production of kibbles with a smaller and more uniform cell structure, resulting in harder kibbles that required a greater force to disintegrate (Dunsford et al., 2002). In this study, rice containing kibbles were harder and tougher than all four sorghum cultivars, while WSB containing kibbles were the closest sorghum variety to the rice containing diet while sumac was the farthest. Future studies should consider analyzing starch gelatinization and the microstructure features of the extrudates through X-ray microtomography to have a better understanding of the relationship between energy input textural properties on extruded pet food. Textural properties can also impact animal preferences, thereby influencing palatability. However, despite having numerical data on kibble texture there is limited published literature on the preference of dogs or cats regarding kibble hardness. Consequently, extrapolation on which of the experimental diets produced in this study a dog or cat would prefer by mouthfeel cannot be made. Yet, when comparing to rice, which is found in nearly half of dry dog and cat food (Plantz, 2018), the WSB and oats diets would have the closest values for hardness and toughness. Comparing the white sorghum varieties and the burgundy sorghum diets, the WSB diet was significantly harder and tougher than the BS diet. The reason may be attributed to the lower screw speed potentially leading to a reduction in starch gelatinization compared to the WSA and BS diets. This did not impact the die pressure upon exiting but may have led to increased hardening during the cooling process.

The total starch content of an ingredient or diet matrix has an impact on the extrusion parameters because extrusion relies heavily on the expansion of the starch granules (Baller et al., 2021). Nitrogen free extract (NFE) has been reported to be a good predictor of total starch in a diet (Alvarenga and Aldrich, 2020). The diets containing rice, WSA and WSB had the highest levels of NFE containing >30% in the final product compared to the other diets which contained 24-27% NFE. Previous studies report that having a high moisture content in extrusion can result in the production of resistant starch (Huang et al., 2022). This may be the reason that oats, which had a much higher water injection into the extruder, resulted in a small dense kibble. Additionally, Huang et al. (2022) reports that a high extrusion temperature can lead to more resistant starch production. Additionally, Alvarenga et al., (2021) reported that the diets that contained more resistant starch were also the diets that had a trend ($P<0.1$) of being the hardest kibbles. In this current study, the diets containing BS and WSB had the highest extrusion temperature and were among the hardest kibbles potentially confirming that resistant starch is present in these diets. Further studies should evaluate the resistant starch content along with degree of cooking of starch in diets and the effect it has on the expansion of diets along with the textural characteristics.

5. Conclusions

The white and burgundy sorghum cultivars (WSA, WSB, and BS) processed well and produced well-formed kibbles, similar to other small grains such as rice, barley, millet, and sumac. Conversely, oats produced small, thin kibbles that may not align with pet owner preferences. Rice, the most common utilized grain in the pet food industry among those evaluated in our study, exhibited the lowest amount of phenolic compounds and total dietary fiber compared to other grains. All four sorghum cultivars evaluated in our study are suitable for substituting rice and the other grains evaluated without compromising processing conditions and kibble quality, with sumac and burgundy sorghum being promising alternatives due to their high phenolic content.

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Table 2-1 Nutritional composition (on a dry matter basis) of the grains used in the study to produce the experimental diets formulated for adult dogs

Nutrient, %	Rice	Barley	Oat	WSA	WSB	BS	Sumac	Millet
Dry matter	88.66	89.45	89.15	89.95	90.07	90.36	89.29	89.86
Organic matter	99.43	98.23	98.11	98.77	98.58	98.23	98.38	95.74
Crude protein	9.46	13.27	14.78	10.23	11.33	11.69	11.92	11.91
Crude fat	1.19	2.55	6.47	3.39	3.58	3.88	3.97	4.32
Total dietary fiber	3.83	17.22	14.69	27.57	25.65	22.69	19.04	27.04
Insoluble fiber	3.27	13.64	10.21	26.79	25.20	22.69	18.82	26.38
Soluble fiber	0.56	3.58	4.37	0.78	0.44	0.0	0.22	0.56
Ash	0.57	1.77	1.89	1.23	1.42	1.77	1.62	4.26

WSA-white sorghum A, WSB- white sorghum B, BS- burgundy sorghum

Table 2-2 Phenolic acid composition and total phenolic content (TPC) of raw grains on a dry matter basis (n=1)

		Grains							
Phenolic content (µg/g flour)		Rice	Barley	Oat	WSA	WSB	BS	Sumac	Millet
	Gallic acid	2.8	16.6	Nq	9.4	6.8	6.3	42.3	21.9
	4-Hydroxybenzoic acid	Nq	Nq	2.7	0.6	4.3	21.8	166.3	2.8
	Vanillic acid	2.2	7.5	9.7	7.5	10.7	20.2	62.7	130.8
	Syringic acid	1	2	29.6	3.2	3.9	10	7.6	19.9
	ρ-coumaric acid	2.3	26.7	69.3	20.5	21.5	20.4	45.2	320.7
	Ferulic acid	45.7	244	160.1	208.5	171.6	236.2	388.1	455.1
	Sinapic acid	1.5	6.9	10.5	Nq	1	Nq	Nq	1.5
	TPC (ug/g)	313	692	903	700	741	1053	3197	2119

Nq- not quantified (peak is too small to quantify)

WSA- white sorghum A, WSB- white sorghum B, BS- burgundy sorghum

Table 2-3 Ingredient and nutritional composition on an as-fed basis of the experimental diets used in this study

Item	Dietary treatments							
	Rice	Barley	Oat	WSA	WSB	BS	Sumac	Millet
Ingredient, %								
Rice	50							
Barley		50						
Oat			50					
White Sorghum A				50				
White Sorghum B					50			
Burgundy Sorghum						50		
Sumac							50	
Millet								50
Chicken Meal	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5
Chicken Fat	8	8	8	8	8	8	8	8
Fish Meal	5	5	5	5	5	5	5	5
Corn gluten meal	5	5	5	5	5	5	5	5
Beet pulp	4	4	4	4	4	4	4	4
Flaxseed	2	2	2	2	2	2	2	2
Dog Digest	2	2	2	2	2	2	2	2
Salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Titanium dioxide	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Potassium chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Mineral premix	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Natural Antioxidant	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Nutritional Composition								
Dry matter, %	93.1	93.4	91.8	92.6	93.6	93.9	91.2	91.8
Crude protein, %	26.6	28.7	29.3	28.8	28.2	29.8	29.2	29.3
Crude fat, %	12.6	14.6	15.8	12.9	14.2	14.1	12.6	15.8
Total dietary fiber, %	10.3	16.7	11.5	9.8	13.0	16.1	15.0	11.5
Insoluble fiber, %	8.0	14.9	7.1	7.7	9.0	13.9	11.4	7.1
Soluble fiber, %	2.3	1.9	4.3	2.1	4.0	2.2	3.5	4.3
Ash, %	8.0	9.2	8.0	8.2	8.2	8.2	6.1	8.0
ME,kcal/kg	3248	3093	3321	3256	3244	3141	3084	3321
NFE, %	35.6	24.2	27.2	32.9	30.0	25.7	28.3	27.2

ME = metabolizable energy, WS A- white sorghum A, WS B- white sorghum B, BS- burgundy sorghum

Calculated ME (kcal/kg)= [(3.5 x % protein) + (8.5 x % fat) + (3.5 x NFE)]x 10

NFE= 100- (crude protein %- crude fat% - total dietary fiber % - moisture%-ash%)

Table 2-4 Nutritional composition of uncoated dried kibble (dry matter basis)

Item %	Rice	Barley	Oats	WSA	WSB	BS	Sumac	Millet	SEM	P-value
Dry Matter	80.54^{ab}	81.69^{a}	74.49^{c}	80.03^{ab}	81.29^{ab}	80.75^{ab}	78.77^{c}	80.24^{ab}	0.820	<0.0001
Organic Matter	89.75	89.83	90.34	90.11	90.96	89.76	90.87	89.70	0.409	0.0286
Crude Protein	33.21^{dc}	35.20^{ab}	36.09^{a}	33.31^{bdc}	32.41^{d}	34.25^{abcd}	34.40^{abc}	32.46^{dc}	0.564	<0.0001
Crude Fat	6.02^{c}	6.68^{bc}	8.95^{a}	7.05^{b}	6.76^{bc}	7.04^{b}	7.45^{b}	6.98^{b}	0.180	<0.0001
Total Dietary Fiber	10.27^{b}	15.27^{b}	14.46^{b}	11.81^{b}	12.87^{b}	15.56^{b}	16.25^{b}	24.51^{a}	1.839	<0.0001
Insoluble Dietary Fiber	7.08^{b}	10.45^{b}	8.72^{b}	8.87^{b}	10.21^{b}	12.34^{b}	11.85^{b}	20.31^{a}	1.818	<0.0001
Soluble Dietary Fiber	3.14^{b}	4.85^{ab}	5.73^{a}	2.99^{b}	2.66^{b}	3.22^{ab}	4.83^{ab}	4.19^{ab}	0.741	0.0062
Ash	10.3	10.2	9.7	9.9	9.0	10.2	9.1	10.3	0.4093	0.0286

^{abcd} Means is a row with unlike superscripts differ ($P<0.05$)

WSA- white sorghum A, WSB- white sorghum B, BS- burgundy sorghum

Table 2-5 Extrusion parameters and kibble characteristics of experimental diets

	Dietary Treatments									
	Rice	Barley	Oats	WSA	WSB	BS	Sumac	Millet	SEM	P-Value
Raw Material										
Feed Rate kg/h	160	160	160	160	160	160	160	160		
Preconditioner										
Discharge Temperature, °C	100^{b}	101^{ab}	101^{ab}	101^{ab}	102^{a}	101^{ab}	101^{ab}	101^{ab}	0.4	0.0294
Steam Injection, kg/h	17.13	17.40	17.67	17.00	17.97	16.73	17.57	17.33	0.352	0.0873
Water Injection kg/h	10.97^{a}	9.70^{c}	10.43^{b}	10.97^{a}	9.97^{c}	10.97^{a}	9.77^{c}	9.67^{c}	0.093	<0.0001
Extruder										
Water injection, kg/h	14.27^{b}	11.43^{cd}	20.33^{a}	14.07^{b}	10.10^{d}	10.57^{d}	12.83^{bc}	12.17^{bcd}	0.631	<0.0001
Extruder screw speed, rpm	454^{ab}	380^{b}	482^{a}	448^{ab}	387^{b}	448^{ab}	380^{b}	380^{b}	23.5	0.0010
Knife speed, rpm	2045^{b}	2046^{b}	2535^{a}	2046^{b}	2046^{b}	2046^{b}	2046^{b}	2046^{b}	0.3	<0.0001
Motor Load	57.3^{a}	55.0^{ab}	42.7^{c}	51.3^{ab}	53.7^{ab}	50.3^{b}	49.3^{c}	51.7^{ab}	2.01	0.0001
Die pressure, PSI	233^{ab}	300^{a}	267^{ab}	233^{ab}	217^{b}	230^{ab}	200^{b}	200^{b}	23.3	0.0081
Die Temp, °C	98^{bc}	109^{a}	100^{b}	96^{c}	107^{a}	98^{bc}	100^{b}	102^{b}	1.3	<0.0001
Kibble Traits- out of the dryer										
Wet bulk density, g/L	316^{d}	387^{b}	514^{a}	349^{c}	357^{bc}	381^{bc}	371^{bc}	374^{bc}	9.2	<0.0001
Bulk Density, g/L	305^{d}	350^{c}	489^{a}	350^{c}	357^{bc}	352^{bc}	366^{b}	343^{c}	4.5	<0.0001

Piece Volume, cm^3	0.57^{a}	0.42^{b}	0.24^{c}	0.50^{ab}	0.50^{ab}	0.57^{a}	0.46^{b}	0.45^{b}	0.027	<0.0001
Piece density, g/cm^3	0.41^{b}	0.46^{b}	0.73^{a}	0.44^{b}	0.47^{b}	0.46^{b}	0.47^{b}	0.46^{b}	0.020	<0.0001
Sectional expansion index	3.65^{a}	3.02^{b}	1.75^{d}	2.89^{b}	2.83^{b}	2.89^{b}	2.33^{c}	2.36^{c}	0.085	<0.0001
Specific length, mm/g	4.93^{ab}	5.36^{a}	4.54^{bc}	4.57^{bc}	4.28^{cd}	3.80^{d}	4.28^{cd}	4.45^{bc}	0.151	<0.0001
Hardness, kg	4.67^{b}	6.68^{a}	3.95^{bcd}	3.46^{cd}	4.24^{bc}	3.38^{d}	2.15^{e}	1.97^{e}	0.265	<0.0001
Toughness, kg*mm	40.20^{b}	57.72^{a}	35.80^{bc}	25.75^{ed}	31.68^{cd}	22.45^{e}	17.85^{ef}	14.09^{f}	2.586	<0.0001

Chapter 3 - The effects of extruded diets containing different sorghum varieties on diet palatability, nutrient digestibility, serum chemistry, hematology and antioxidant capacity in healthy adult dogs

Abstract

Sorghum varieties with high polyphenol content, such as burgundy sorghum, have the potential to provide health benefits to dogs due to their antioxidant properties. However, there are no published studies on the evaluation of the apparent total tract digestibility (ATTD), plasma antioxidant capacity or palatability of dogs fed diets containing burgundy sorghum cultivar compared to the traditional white sorghum. Therefore, the objective of this study was to determine these attributes on extruded diets containing 50% of white sorghum (two different varieties; A and B), burgundy sorghum or rice (positive control) in adult healthy dogs. Twelve adult Beagles (6 male and 6 females; 12.8 ± 1.7 kg) were randomly assigned to treatments and individually housed. Dogs were fed twice daily in periods that included a 9-d diet adaptation followed by a 5-d fecal collection in a replicated 4x4 Latin Square design. At the end of each period, fasted blood samples were collected for serum metabolites, hematology and antioxidant capacity analyses. Data were analyzed using the GLIMMIX procedure of SAS 9.4 There were no differences ($P > 0.05$) in crude protein and crude fat ATTD among dietary treatments. Dogs fed the rice diet had greater ($P > 0.05$) organic matter ATTD than dogs fed the diets containing sorghum. Dry matter and gross energy ATTD were similar ($P > 0.05$) in dogs fed the rice and white sorghum A diet, but lower ($P < 0.05$) for the dogs fed white sorghum B and burgundy sorghum compared to those fed the rice diet. Fecal score evaluation indicated no influence ($P > 0.05$) of diet on fecal consistency. In the palatability assessment, no preference ($P > 0.05$) was observed for the burgundy compared to white sorghum A diet; however, dogs preferred ($P < 0.05$) the white sorghum A over the white sorghum B diet. When the former was presented simultaneously with the rice diet, the dogs approached the rice diet first ($P < 0.05$), but no differences in intake were observed ($P > 0.05$). The rice diet was approached first by dogs when presented with the white ($P< 0.05$). No influence ($P > 0.05$) of diet on antioxidant capacity of fasted plasma in dogs was observed, suggesting that sorghum bran extract may be necessary to boost the antioxidant potential of dogs. Dogs fed diets containing sorghum, regardless of the variety, had lower ($P < 0.05$) plasma cholesterol levels compared to those fed the rice diet. Overall, our results indicate that sorghum containing diets are well tolerated by dogs and may lower circulating cholesterol levels, and had slightly lower nutrient OM digestibility but comparable CP ATTD compared to rice when fed to healthy adult dogs.

Keywords: Burgundy sorghum, white sorghum, functional ingredients, extruded pet food

1. Introduction

Sorghum is an ancient grain with a potential to be included at the expense of traditional cereal crops in extruded pet food due to its sustainability and functionality attributes. In terms of food processing, sorghum can be included at high levels in extruded diets without drastically affecting processing conditions (Alvarenga et al., 2018). Sorghum may also be an attractive ingredient option for the pet food industry due to the high concentration of polyphenols in some sorghum varieties. The dietary intake of polyphenols, which are secondary metabolites of plants involved in defensive system to insect predation, may provide cellular protection against oxidative stress and inflammation due to their antioxidant properties (Scott et al., 2022, Aravind et al., 2021). Sorghum's polyphenols are mostly found in the pericarp (Tanwar et al., 2023), with darker pericarp colors having greater concentrations of polyphenols (Davis et al., 2019). To act as antioxidants in the extra splanchnic metabolism, it is vital that polyphenol compounds are absorbed through the gut barrier and reach the bloodstream (Scott et al., 2022). While small phenols are easily absorbed in the small intestine, bigger ones such as proanthocyanidins, which are commonly found in sorghum, are poorly absorbed (Scott et al., 2022). Additionally, food processing and the food matrices in which polyphenols are included can impact their bioavailability (Scott et al., 2022). While the bioavailability of polyphenols has been more extensively studied in rats and humans (El Khawand et al., 2018, Scholz and Williamson, 2007, Wiseman, 1999, Jain et al., 1999, Masisi et al., 2016, Vissers et al., 2004), there is a lack of data on bioavailability of polyphenols in extruded dog diets. The published studies of antioxidants in canines primarily focus on the effects of supplementation of more purified antioxidants on oxidative stress and inflammatory responses rather than ingredients that inherently possess antioxidant potential (Hall et al., 2006, Heaton et al., 2002, Martello et al., 2023). Moreover, while multiple studies have evaluated the apparent total tract digestibility (ATTD) of diets containing sorghum in adult dogs (de Silva Junior et al., 2005, Fortes et al., 2010, Bazolli et al., 2015, Twomey et al., 2003, Carciofi et al., 2008, Kore et al., 2009, Duarte et al., 2005), to our knowledge, none have evaluated how different sorghum varieties affect nutrient digestibility and antioxidant status in healthy dogs. We hypothesized that the palatability and the ATTD of macronutrients would be similar among dietary treatments, but that the burgundy sorghum diet would yield greater fasted oxygen radical absorbance capacity (ORAC) in the plasma. Therefore, the objectives of this study were to determine the palatability, ATTD of macronutrients and energy, and antioxidant capacity of three diets containing different sorghum cultivars (two white varieties and one burgundy (BS) compared to a rice-based diet (control) when fed to healthy Beagles.

2. Materials and Methods

2.1 Diet Formulation and Production

Four nutritionally complete and balanced diets were formulated to meet or exceed the nutritional recommendations for adult dogs at maintenance set by the Association of American Feed Control Officials (AAFCO, 2023). Each dietary treatment was formulated to include either white sorghum A (WSA), white sorghum B (WSB), BS sorghum or rice at 50 % inclusion on an as-fed basis (Table 3-1). The nutritional composition of the grain utilized in this study is presented in Chapter 2. Sorghum varieties were sourced from a regional purveyor (NuLife Market, Scott City, KS) and rice was sourced from a broker (Anchor Ingredients, Fargo, ND). The white sorghum varieties were selected based on their expected differences in starch and total dietary fiber content; however, the proximate analysis following the study revealed that they were similar in their nutrient composition (Chapter 2). The remaining 50% of the diet was composed of a basal mix sourced from a local mill (Fairview Mills, Seneca, KS) with chicken fat and dog digest applied topically after extrusion. The chicken fat was supplied by a renderer (International Dehydrated Foods; Monett, MO) and the canine digest flavor by a flavoring company (AFB International; St. Charles, MO). The diets were extruded on two separate days using a single screw extruder (Wenger Manufacturing Inc, Sabetha, KS) with a 6 mm die diameter (Chapter 2). Titanium dioxide was added to the diet at 0.4% as an external marker to calculate ATTD.

2.2 Palatability Assessment

All dietary treatments were evaluated against the WSA diet in a two-bowl test using a trained dog panel (Summit Ridge Farms, Susquehanna, PA). In the procedure over two consecutive days, stainless steel bowls each containing approximately 400 g of each food were presented simultaneously to the dogs for 30 min. To prevent side bias, bowl position was switched on the second day. First choice was recorded by technicians and intake ratio (IR) was calculated as follows:

$$IR = \frac{consuption\ of\ diet\ A}{(consumption\ of\ diet\ A + consumption\ of\ diet\ B)}$$

2.3 Apparent Total Tract Digestibility Assessment

The Institutional of Animal Care and Use committee at Kansas State University approved all animal procedures (Protocol No. 4097). Twelve healthy adult Beagles (6 females and 6 males), with an average weight of 12.8 ± 1.7 kg, were enrolled in the feeding study. The study was conducted as a 4×4 Latin square design with each dietary treatment randomized to each dog and period according to Kim and Stein (2009). For each period, dogs were fed experimental diets for 9 days of adaptation followed by 5 days of fecal collections.

The dogs were individually housed in a climate-controlled building at 22-23 °C with 16 h light and 8-h dark cycle. Dogs were fed twice daily at 0800h and 1700h and water was available *ad libitum* throughout the study. Food intake was provided to maintain body weight and was determined based on maintenance energy requirement calculated according to the National Research Council (NRC, 2006). The metabolizable energy (ME) of the dietary treatments was calculated using modified Atwater factors. During the collection period, all feces and orts were collected and weighed daily. Fecal samples were collected, weighed, and scored on a scale of 1 to 5 in 0.5 increments (where 1 is liquid diarrhea and 5 is considered dry, hard pellets). A score of 3.5-4.0 was considered ideal. Fecal samples were stored at -20˚C in whirl-pak bags labeled with the dog's name and date.

2.4 Apparent Total Tract Digestibility Calculation

After each collection period, feces were dried at 55 °C in a forced air oven for 24-48 h. Dried feces were then ground to 1 mm using a hammer mill (Wiley Mill Model 3, Swedesboro, NJ.) for analysis. Apparent total tract digestibility was calculated as follows:

$$\text{ATTD} = [1 - \frac{(\%\text{TiO}_2 \text{ in food*\% nutrient in feces})}{(\%\text{ TiO}_2 \text{ in feces*\% nutrient in food})}]*100$$

2.5 Nutrient Analysis

Diet samples and partially dried fecal samples were analyzed for moisture (AOAC 930.15), ash (AOAC 942.05), fat by acid hydrolysis (ISO 11085:2008), crude protein (CP) as nitrogen (AOAC 990.03) using a nitrogen analyzer (FP928, LECO Corporation, Saint Joseph, MI), gross energy (Parr 6200 Calorimeter, Parr Instrument Company, Moline, IL), and total dietary fiber (TDF) (AOAC 991.43).

2.6 Blood collection

Prior to the start of the study and on the last day of each experimental period, fasted blood was collected (approximately 6 mL) from each dog through cephalic venipuncture and a sample (approximately 3 mL) was immediately transferred into an EDTA tube for analyses. The remaining blood sample was transferred to heparin vacutainer tubes that were chilled on ice until they were centrifuged at 2000 × G for 10 minutes to separate and harvest plasma. Whole blood and plasma samples were immediately submitted to Kansas State University Diagnostic Laboratory for serum biochemistry and hematology analysis. The remaining samples were then stored in a -80 °C until antioxidant capacity was analyzed.

2.7 Oxygen Radical Absorbance Capacity Analysis

Dog plasma was analyzed for oxygen radical absorbance capacity (ORAC) using a commercial kit (Cell Biolabs Inc: CA, USA). Sample preparation and fluorescence procedures were performed according to the kit protocol. Fluorescence was recorded every minute for 60 min at excitation and emission wavelengths of 480 and 520 nm, respectively, using a plate reader (Gen5; Biotek Instruments, Inc. Winooski; VT, USA). Results were calculated according to the standard curve (Gen5 Microplate Data Collection and Analysis Software) with an intra coefficient of variation of 5.5%.

2.8 Statistical Analysis

Apparent total tract digestibility of macronutrients and energy, and blood ORAC, hematology and biochemistry data were analyzed using the GLIMMIX procedure in SAS (SAS v. 9.4). Treatment was used as a fixed effect, and dog and period were considered random effects. Tukey's post hoc test was used to separate least square means when significant was found at $P < 0.05$. Fecal scores were analyzed as categorical data assuming a multinomial distribution. The data were analyzed using the GLIMMIX procedure in SAS (SAS v. p.4) with dog and period as random effects and treatment as a fixed effect. In the palatability study, IR was analyzed using a t-test in a two-way ANOVA and first choice was analyzed using a chi-square test.

3. Results

3.1 Diet nutritional composition and dog's intake, output, and fecal parameters

The nutritional composition of the experimental diets was similar for most nutrients (Table 3-1). Dry matter (DM) content of the diets ranged from 93 to 94% and the ash content was approximately 8% (DM). Minor differences were observed for the CP and TDF content of the diets. Crude protein was lower in the rice containing diet (29% DM) compared to the sorghum-containing diets (30-31% DM). The rice and WSA containing diets contained approximately 14% fat (DM), while the WSB and BS containing diets contained 15% (DM). The TDF content of the experimental diets differed the most, with the lowest concentration observed in the WSA and rice containing diets (11% DM) followed by the WSB (14% DM) and the BS-containing (17% DM) diets. These differences were mostly driven by the insoluble fiber content.

Food intake was similar (P > 0.05) among dietary treatments (Table 3-2). Energy intake (kcal ME/day) was greater (P < 0.05) in dogs fed the WSB containing diet compared to the dogs fed the BS containing diet. However, no differences in body weight were observed. No differences were observed in wet fecal output, defecations per day, or fecal pH (Table 3-2; P > 0.05). Additionally, when looking at the frequency of the fecal scores no differences were observed (P > 0.05; Figure 3-2).

3.2 Apparent total tract digestibility

Dry matter and energy ATTD were similar (P > 0.05) among dogs fed the WBA and the rice containing diets (87% vs. 86% DM ATTD and 90 vs. 89% gross energy ATTD), but they were greater (P < 0.05) in dogs fed the rice diet compared to those fed the WSB and the BS containing diets (84% vs. 85 % for dry matter ATTD vs. 88% and 88% ATTD; Table 3-2). Dogs fed the rice containing diet had greater (P < 0.05) organic matter ATTD (90%) compared to those fed the sorghum containing diets (87.8 %). No differences (P > 0.05) were observed in CP and fat ATTD among dogs consuming the experimental diets.

3.3 Blood parameters and antioxidant capacity

No differences (P > 0.05) were observed in the hematological parameters among dogs fed the experimental diets, with exception of red blood cell distribution width and anion gap (Table

3-3). Red blood cell distribution was greater ($P < 0.05$) in dogs fed the WSA diet compared to those fed the WSB and BS diets. The anion gap was greater ($P < 0.05$) in dogs fed the rice diet compared to those fed the WSA diet. However, all values for red blood cell distribution and anion gap were within the reference range. Dogs fed the rice-based diet had greater ($P < 0.05$) cholesterol levels than those fed the sorghum containing diets, which were similar ($P > 0.05$) among each other. No statistical differences were observed in the other hematological and serum biochemistry parameters. The average of creatine kinase serum levels was above the reference range for dogs fed the sorghum containing diets, which was driven by ten dogs. Three observations of elevated alkaline phosphatase levels were attributed to a single dog when consuming the sorghum-based diets. Cholesterol had one observation that was higher than the reference range when fed the rice diet. Glucose had seven observations driven by six dogs that were below the reference range when fed all four diets, while six of those specified observations were in period one. In addition, there were several parameters that had dogs slightly above or below the reference range, however, the numerical differences from the reference range were minimal that it was not to warrant concern. These parameters included sodium, total protein, albumin, globulin, calcium, potassium, chloride, bicarbonate, anion gap, and the sodium potassium ratio. No differences ($P < 0.05$) were observed in the fasted plasma ORAC values among dogs fed the experimental diets.

3.4 Palatability

When the WSA and the BS based diets were simultaneously presented to dogs, no preference ($P > 0.05$) was observed for first choice or intake ratio (Table 3-5). However, dogs consumed more ($P < 0.05$) and approached the WSA diet first ($P < 0.05$) when it was offered with the WSB diet. When comparing the WSA to the rice diet, dogs approached the rice diet first (28 vs. 12 times; $P < 0.05$), but diet consumption indicated by the IR did not differ ($P > 0.05$).

3. Discussion

To the author's knowledge, this is the first study published to evaluate and compare the inclusion of BS and white sorghum varieties in expanded pet food and their impact on diet palatability, nutrient digestibility, blood metabolites and antioxidant capacity when fed to healthy adult Beagles. An experimental diet containing rice added at the same inclusion level as sorghum was included in the study as rice is a common grain utilized in commercial extruded dog food. The nutritional composition of the diets was relatively similar with differing the most in total dietary fiber content. Because the diets had similar nutritional composition, we are more confident in attributing the results to the ingredient differences between diets.

While digestibility and blood constituent results are important for the validation of an ingredient and its utility and health benefits, palatability is an important attribute that impacts the commercial success of the product and the voluntary ingestion by the animal (Aldrich and Koppel, 2015). Sorghum is typically described to have a bitter taste, however, previous research in the pet food industry has not shown an issue with sorghum palatability (Pezzali et al., 2021, Lema Almeida et al., 2022). In our study, there was no difference in the intake ratio between the WSA and rice containing diets. Conversely, the WSB containing diet was preferred less when compared to the WSA containing diet. Total dietary fiber and fat composition did not differ between the diets, so the lack of palatability in the WSB containing treatment may be due to texture or flavor from phenolic compounds (Kobue-Lekalake et al., 2007). When the BS containing diet intake ratio was compared to WSA containing diet, no differences were observed.

Pezzali et al. (2021) reported that cereal bars containing red sorghum and white sorghum crisp had the same acceptance compared to cereal bars produced with rice crisp in dogs. While the WSB containing diet did not perform well in the split bowl test, during the feeding trial there was no refusal, and therefore, diet acceptance was not an issue when substituting sorghum for rice in dog diets. We do acknowledge that certain sorghum cultivars may be more palatable than others. This has not previously been described as it relates to pet foods.

In this study, dog food intake was metered to assure they maintained body weight. This amount is slightly less than they would prefer to consume *ad libitum* and so there were no refusals and since each dog serves as its own control no differences in intake across treatments was observed. Variations in daily energy intake (kcal/d) observed were a result of variations in metabolizable energy (ME) content in diets and adjustments in feeding quantities to maintain body weights. This is consistent across similarly designed studies (Kore et al., 2009) and Alvarenga and Aldrich (2018) wherein no differences in intake were observed when a substantial percentage of the diet was derived from sorghum (>50%). Additionally, there were no differences in fecal output (grams per day) or frequency (number or defecations per day) which was expected since the TDF did not vary substantially among diets (6%). Additionally, because of the small percentage difference in total dietary fiber, it was hypothesized that no differences in fecal scores would be observed. This was confirmed in our study as the quality of the fecal scores were not different among treatments. Previous studies that also evaluated sorghum compared to other grains (corn, rice, millet, peas) and observed no differences in fecal scores when sorghum was included at >50% (Kore et al, 2009, Bazolli et al, 2015, Carciofi et al, 2008). This affirms visually and qualitatively that sorghum-based diets are being digested and tolerated well by dogs.

Sorghum has had a negative perception due to its reputed bitter taste and low protein digestibility. This unfavorable reputation is rooted in benchtop tests or livestock studies conducted several decades ago (Duodu et al., 2003, McCollough and Brent, 1972). In the current study there were no differences observed among diets containing sorghum cultivars in fat or protein digestibility. This confirms the report from von Schaumburg (2021) who found similar fat and protein digestibility coefficients in red and white sorghum-based cat diets. Additionally, there were no differences among sorghum treatments and the rice containing diet in protein digestibility. Again, in agreement with previous studies that utilized over 50% sorghum and reported protein digestibility exceeding 80% (Twomney et al., 2003, Silva Junior et al., 2005, Carciofi et al., 2008, Kore et al., 2009, Bazolli et al., 2015, Alvarenga and Aldrich, 2018). These works further suggest that in dog and cat diets, sorghum has acceptable protein digestibility. This is likely attributable to processing by extrusion which helps reduce antinutritional factors, such as phytate, trypsin inhibits, and oxalates that can be found in Sorghum (Nikmaram et al., 2017). In our work, all sorghum cultivar-based diets had high nutrient ATTD (>85%) with the rice containing diet being superior compared to the diets containing WSB and BS only in organic matter and gross energy ATTD. However, the differences were all within 3% of each other and all above 87% digestible, confirming high digestibility in all diets.

Assuming no degradation during extrusion and no addition of polyphenols from other ingredient sources, our diets are expected to have 157-527 µg/g (dry matter) of total phenolics based on the total phenolic content of the raw ingredients (Chapter 2; rice being the lowest and BS the highest). Polyphenols are poorly absorbed in the small intestine (5-10%) which may provide benefit for gut health (Scott et al., 2022). Polyphenols may act as prebiotics by affecting (increasing) beneficial microbes in the colonic ecosystem, while reducing the nutrients available for harmful bacteria such as E. Coli and Salmonella (Aravind et al. 2021). Additionally, Ashley et al. (2019) found that sorghum extracts (sumac sorghum and black sorghum) were effective at maintaining gut homeostasis and elevating beneficial populations such as *Bifidobacterium* and *Lactobacillus*. Unfortunately, we were unable to evaluate volatile fatty acids and other fecal markers of gut health in this study. Future studies should explore the effect of sorghum polyphenols on markers of gut health in dogs.

While only 5-10% of polyphenols are absorbed in the small intestine, many studies suggest that this percentage is sufficient for antioxidant effects (i.e. anticancer, antidiabetic, anti-inflammatory effects) to occur. Antioxidants are one of the attractive benefits sorghums possesses. While it was anticipated that dogs fed the BS containing diet would have greater fasted plasma ORAC values than the ones fed the white sorghum cultivars and rice containing diets because of the greater phenolic content of BS, this was not observed in our study. Alvarenga and Aldrich (2018) also did not observe differences in the fasted plasma ORAC values of dogs fed a diet containing whole sorghum (64% inclusion) compared to a sorghum flour-based diet (63% inclusion) and a non-sorghum-based control. Additionally, the sorghum-mill feed treatment (67% inclusion), which has greater content of polyphenols compared to whole sorghum and sorghum flour, did impacted the plasma ORAC values compared to the other sorghum containing diets (Alvarenga and Aldrich, 2018). To investigate the effects of diets with varying levels of polyphenols on the antioxidant capacity of fasted plasma samples, one might consider subjecting dogs to a stress event (e.g. reduced feeding, high levels of exercise, transportation). Additionally, the evaluation of antioxidant capacity in the fed state might be beneficial to provide insights on the ability of dogs to absorb the polyphenols present in diets containing BS sorghum. In healthy adults, a greater plasma antioxidant capacity was observed at

2 h post-prandial compared to fasted samples when the participants consumed pasta containing red sorghum flour (30%) compared to those who consumed pasta containing white sorghum or wheat flour (Khan et al., 2014). In a study conducted by Torabian et al. (2009), it was reported that antioxidant capacity peaked at 150 min post-feeding while plasma polyphenols peaked and stayed steady from 90 minutes post-feeding to 210 minutes post-feeding. Furthermore, Hudthagosol et al. (2011) also reported that ORAC values increased by 10-12% at 2 hours post-feeding. Therefore, future studies should investigate the post meal antioxidant and anti-inflammatory response of dogs fed antioxidant rich diets. Additionally, sorghum bran extracts hold promise to deliver higher inclusions of polyphenols in extruded diets, and thus, to have an effect on the antioxidant status of dogs. In a study conducted by Devi et al. (2011), red sorghum bran extract concentrated with anthocyanins (15 ug/ml to 1000 ug/ml) was used to inhibit the growth of human breast cancer cells in vitro. Additionally, in rats with lung cancer, a procyanidin-rich sorghum bran extract significantly restored D-galactose induced oxidative stress (150 mg/kg dose) and inhibited the tumor growth and metastasis formation (200 and 40 mg/kg doses; Wu et al., 2011). However, in both experiments, the extracts were administered intravenously rather than orally through the food matrix. During extrusion, food is subjected to high heat and pressure, which can have an impact on the survivability of the antioxidants (Sarka et al., 2021, Brennan et al., 2011). Retention of polyphenols during extrusion has been studied previously, mainly in the human food industry, with conflicting outcomes based upon the type of extruder, extruder parameters, and grain type (Sarka et al. 2021, Brennan et al., 2011). Thus, it is important that future studies evaluate the effect of extrusion on the sorghum polyphenols, their bioavailability after processing and impact on antioxidant capacity in dogs. Furthermore,

sorghum extracts may be a good addition to raw, fresh, or freeze dried pet foods which do not undergo thermal processing.

In addition to ORAC, blood was analyzed for hematology and serum profiles to evaluate the overall health of the dogs in the study. While many of the blood parameters were outside the reference range for some dogs, all dogs remained healthy throughout the study and all blood parameters were sufficiently close to the reference range to not raise concerns. However, one parameter that exhibited a significant number of observations outside the reference range and deviated further from it was glucose. Yet, six of the seven observations that were out of the reference range were in period one, and the vast majority of the dogs had plasma glucose within reference range continuously throughout the rest of the trial. This may suggest an error in the laboratory analysis. The experimental diets influenced two hematological parameters, anion gap and red blood cell distribution; however, the differences were minimal to have a physiological effect. Anion gaps and red blood cell distribution widths typically do not attract attention unless they deviate from the reference range (Kraut and Madias, 2007, Danese et al., 2015), a circumstance that did not occur in this study. The experimental diets influenced only the cholesterol levels among all analyzed serum metabolites, with dogs consuming sorghum-based diets showing lower plasma cholesterol concentrations compared to those fed the rice-based diet. Studies in hamsters have reported lower cholesterol levels when sorghum extracts or sorghum oil where provided, which is hypothesized to be due to phytosterol and/or policosanol rich oil or wax (Lee et al., 2014 with 5% sorghum oil inclusion, Carr et al., 2005 with 0-5% sorghum oil inclusion). Phytosterols are plant-based sterols that have been linked to lowering cholesterol (Ostlund Jr, 2004, Nguyen 1999). Sorghum (Cargill 727 and 888Y varieties) has been reported to have high levels of free phytosterols and fatty acyl phytosterol esters (Singh et al., 2003). Canine elevated cholesterol, or hypercholesterolemia, can be caused by both primary or secondary factors, the latter being more predominant (Xenoulis and Steiner, 2010). Secondary factors include endocrine disorders, pancreatitis, protein-losing nephropathy, obesity and others. While hypercholesterolemia seems to be of less clinical importance than high levels of triglycerides in dogs, severe hypercholesterolemia may be treated with ultra-low-fat diets. It is also not uncommon for both hypercholesterolemia and hypertriglyceridemia to be observed in dogs, where dietary management is important. Inclusion of sorghum in ultra-low-fat diets for the management of hyperlipidemia may be a good alternative. Diabetic rats, whether diet-induced or streptozotocin-induced, have been reported to have reduced plasma triglycerides levels when fed sorghum extracts (Kim and Park, 2012, 400 and 500 mg/kg dose, Chung et al., 2011, 100 and 250 mg/kg dose, Chung et al., 2010, 50 and 300 mg/kg dose). However, research investigating the effects of sorghum on lipid levels in dogs with hyperlipidemia is necessary to confirm this hypothesis as our study was conducted in healthy dogs with cholesterol levels within the physiological range.

4. Conclusion

Diets including BS sorghum and different white sorghum cultivars were highly digestible by adult dogs and had no negative effects on fecal quality. Dogs preferred diets with the inclusion of WS-A compared to those containing WSB, while rice-based diet was chosen first by dogs when presented with the WS-A diet, but with no changes on food intake. Despite these differences, there were no refusals during the digestibility feeding trial with all diets being well accepted by the dogs. Dietary treatments had minimal effects on the serum metabolites and hematological parameters evaluated. However, dogs fed the sorghum-based diets had lower levels of plasma cholesterol compared to those fed the rice-based diet, which may be beneficial for dogs with hypercholesterolemia, a rare condition. There were no differences in fasted plasma antioxidant capacity among dogs fed the sorghum and rice-based diets, despite the greater total phenolic content of BS sorghum, followed by the white sorghum varieties and rice. Future studies should consider evaluating the post-prandial antioxidant status of dogs fed sorghum-based diets and the use of a sorghum bran extract to achieve a greater dietary inclusion of sorghum polyphenols. Fecal scores, output, and frequency were similar among all treatments. No differences were observed in crude protein or fat digestibility among treatments although the rice-based diet had a higher organic matter and gross energy ATTD compared to the sorghum-based diets. Overall, the sorghum-based diets provide a good substitution for rice in an extruded diet fed to healthy adult dogs.

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Table 3-1 Ingredient and nutritional composition of the experimental diets used in this study

Item	Rice	WSA[1]	WSB	BS
Ingredient, % (as-fed basis)				
Rice	50			
White sorghum A		50		
White sorghum B			50	
Burgundy sorghum				50
Chicken Meal	22.5	22.5	22.5	22.5
Chicken Fat	8	8	8	8
Fish Meal	5	5	5	5
Corn gluten meal	5	5	5	5
Beet pulp	4	4	4	4
Flaxseed	2	2	2	2
Dog Digest	2	2	2	2
Salt	0.4	0.4	0.4	0.4
Titanium dioxide	0.4	0.4	0.4	0.4
Choline chloride	0.2	0.2	0.2	0.2
Potassium chloride	0.2	0.2	0.2	0.2
Vitamin premix	0.15	0.15	0.15	0.15
Mineral premix	0.1	0.1	0.1	0.1
Natural Antioxidant	0.05	0.05	0.05	0.05
Nutritional composition and energy content (dry matter basis)				
Dry matter, %	93.1	92.6	93.6	93.9
Crude protein, %	28.6	31.1	30.1	31.8
Crude fat, %	13.6	14.0	15.2	15.0
Total dietary fiber, %	11.1	10.6	13.9	17.2
Insoluble fiber, %	8.6	8.3	9.6	14.8
Soluble fiber %	2.5	2.3	4.3	2.3
Ash, %	8.0	8.2	8.2	8.2
Nitrogen-free extract, %	31.8	28.7	26.2	21.7
ME[2], kcal/kg	3270	3283	3263	3148

[2] Calculated ME (kcal/kg) = [(3.5 x % protein) + (8.5 x % fat) + (3.5 x NFE)] x 10

[1] WSA- white sorghum A, WSB- white sorghum B, BS- burgundy sorghum

Table 3-2 Food intake, fecal parameters, and apparent total tract digestibility (ATTD) of dogs fed the experimental diets containing different sorghum varieties or rice

Item	Rice	WSA[1]	WSB	BS	SEM	P-value
Intake (g/d, as-is)	192	193	195	192	7.5	0.7160
Energy intake (kcal ME /d)	602^{ab}	605^{ab}	613^{a}	586^{b}	23.2	0.0249
Body weights, kg	13.2	12.9	13.2	13.1	0.12	0.2090
Fecal Output (g/d, as-is)	50.9	58.7	65.6	67.8	7.9	0.1527
Defecations per day	1.25	2.51	1.28	1.53	0.997	0.5507
Fecal pH	5.94	5.63	5.77	5.66	0.151	0.1729
ATTD, %[2]						
Dry Matter	87.0^{a}	85.5^{ab}	84.5^{b}	84.0^{b}	0.86	0.0082
Organic matter	90.4^{a}	88.5^{b}	87.8^{b}	87.0^{b}	0.69	0.0002
Crude protein	86.9	86.7	85.2	85.5	0.78	0.0981
Acid-hydrolyzed fat	96.9	96.7	96.7	96.6	0.18	0.3063
Gross energy	90.1^{a}	89.0^{ab}	88.2^{b}	87.7^{b}	0.63	0.0037

[1] WSA- white sorghum A, WSB- white sorghum B, BS- burgundy sorghum

[2] ATTD was determine using titanium dioxide as an external marker

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

Table 3-3 Fasted hematology profile in plasma of healthy adult dogs fed experimental diets containing different sorghum varieties or rice

Hematological parameter	Treatments				SEM	P-value	Reference Interval[2]
	Rice	WS[1]	WSB	BS			
Hemoglobin (g/dL)	18.5 (15.6-20.1)	18.6 (14.7-21.5)	18.2 (15.5-20.4)	18.4 (16.8-20.1)	0.34	0.6455	14.1-20.5
Hematocrit (%)	55 (46-60)	55 (44-62)	54 (45-60)	54 (50-58)	0.9	0.4673	41-59
MCV (fL)	71.9 (69.6-75.7)	71.6 (68.0-75.2)	71.9 (68.0-74.1)	71.8 (68.2-74.4)	0.16	0.1722	64.0-76.0
MCH (pg)	24.4 (23.2-25.1)	24.3 (23.2-25.0)	24.2 (22.9-25.3)	24.3 (23.4-25.5)	0.10	0.3612	22.0-26.0
MCHC (g/dL)	34.0 (32.9-34.8)	34.0 (33.1-34.9)	33.7 (32.8-35.0)	33.9 (32.8-34.9)	0.11	0.0997	33.0-36.0
RDW (%)	12.8^{a} (12.1-13.6)	12.9^{a} (11.9-13.6)	12.7^{b} (12.0-13.3)	12.8^{ab} (12.3-13.6)	0.06	0.0179	11.4-13.7
Platelet (electronic) (10^{3uL})	200 (173-253)	213 (157-282)	212 (147-262)	218 (165-210)	8.1	0.2538	130-370
MPV (fL)	12.7 (11.0-14.1)	13.8 (10.8-14.2)	12.8 (10.4-14.1)	12.1 (10.8-27)	0.93	0.3464	8.3-15.3

Segmented Neutrophils (10^3/uL)	4.6 (3.0-6.1)	4.4 (3.3-6.0)	4.5 (3.1-5.2)	4.5 (3.0-6.1)	0.21	0.9306	2.5-9.3
Lymphocytes (10^3/uL)	1.4 (0.6-2.6)	1.4 (0.8-1.9)	1.3 (0.8-2.6)	1.5 (1.1-2.0)	0.14	0.4047	0.8-4.3
Monocytes (10^3/uL)	0.3 (0.1-0.7)	0.2 (0-0.6)	0.3 (0-0.7)	0.3 (0-0.5)	0.06	0.6030	0.1-0.9
Eosinophils (10^3 /uL)	0.2 (0-0.6)	0.2 (0-0.4)	0.1 (0-0.4)	0.1 (0-0.4)	0.05	0.6367	0.0-1.5
Basophils (10^3 /uL)	0	0	0	0			0.0-0.1

[ab] Means within a row with unlike superscript differ ($P<0.05$)

[1] WSA- white sorghum A, WSB- white sorghum B, BS- burgundy sorghum

MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RDW, red blood cell distribution width; MPV, mean platelet volume

[2] Reference interval for hematology profile was determined by Kansas State Veterinary Diagnostic Laboratory using 129 dogs of mixed breeds and ages

Numbers in parentheses are the minimum and maximum values observed for each parameter

Table 3-4 Fasted serum biochemistry profile in plasma of healthy adult dogs fed experimental diets containing different sorghum varieties or rice

Serum metabolite	Treatments				SEM	P-value	Reference Interval[2]
	Rice	WSA[1]	WSB	BS			
Alanine transaminase P5P (U/L)	44 (24-67)	41 (21-74)	38 (19-66)	44 (23-101)	5.0	0.6711	20-144
Albumin (g/dL)	3.9 (3.6-4.1)	3.9 (3.5-4.2)	3.9 (3.6-4.3)	3.8 (3.5-4.3)	0.04	0.1685	3.2-4.2
Alkaline phosphatase (U/L)	42 (14-125)	43 (11-168)	42 (12-169)	44 (15-165)	4.5	0.9563	10-130
Anion gap, (mmol/L)	24.6^{a} (20-28)	22.8^{b} (17-27)	23.8^{ab} (19-29)	23.1^{ab} (20-27)	0.61	0.0314	18-27
Bicarbonate (mmol/L)	17.5 (13-22)	18.9 (17-21)	18.3 (14-21)	18.5 (16-19)	0.69	0.2274	18-24
Bilirubin, total (mg/dL)	0.1 (0.1)	0.1 (0.1)	0.1 (0.1-0.2)	0.1 (0.1-0.2)	0.01	0.7487	0.0-0.2
Calcium, total (mg/dL)	10.5 (9.9-11.7)	10.5 (9.5-11.4)	10.5 (9.7-11.4)	10.4 (9.6-11.0)	0.08	0.2188	9.5-11.2
Chloride (mmol/L)	110 (107-113)	111 (108-114)	110 (107-114)	110 (105-113)	0.4	0.2941	106-117
Cholesterol (mg/dL)	246^{a} (147-405)	227^{b} (159-320)	223^{b} (156-343)	225^{b} (166-314)	5.5	0.0007	140-390
Creatine kinase (U/L)	217 (155-315)	240 (126-486)	246 (160-436)	259 (163-395)	30.7	0.5894	54-226
Creatinine (mg/dL)	0.7 (0.5-1.1)	0.8 (0.4-1.0)	0.7 (0.5-0.9)	0.7 (0.5-0.9)	0.02	0.6219	0.6-1.4

Globulin, (g/dL)	2.4 (2.2-3.1)	2.4 (2.1-2.8)	2.5 (2.0-3.2)	2.5 (2.1-2.9)	0.07	0.8831	1.8-3.0
Glucose (mg/dL)	82 (61-100)	83 (63-100)	83 (63-94)	82 (66-99)	2.0	0.9415	70-120
Phosphorous (mg/dL)	3.8 (3.1-5.0)	3.7 (2.8-4.8)	3.9 (3.3-4.4)	3.6 (2.7-4.6)	0.19	0.6142	2.2-6.1
Potassium (mmol/L)	4.6 (4.3-3.9)	4.7 (4.2-5.0)	4.6 (4.3-5.0)	4.6 (4.1-5.2)	0.07	0.6320	3.7-5.0
Protein, total (g/dL)	6.3 (5.8-7.2)	6.3 (5.7-6.9)	6.4 (5.7-7.0)	6.3 (5.9-7.2)	3.23	0.3427	5.3-6.9
Sodium (mmol/L)	147 (142-149)	147 (141-150)	147 (144-149)	146 (143-151)	0.3	0.0976	144-151
Urea nitrogen (mg/dL)	14 (11-18)	15 (10-20)	15 (10-18)	15 (9-20)	0.6	0.1974	8-29

[ab] Means within a row with unlike superscript differ ($P < 0.05$)

[1] WSA- white sorghum A, WSB- white sorghum B, BS- burgundy sorghum

[2] Reference interval for serum metabolites was determined by Kansas State Veterinary Diagnostic Laboratory using 121 dogs of mixed breeds and ages

Numbers in parentheses are the minimum and maximum values observed for each parameter

Table 3-5 First choice and intake ratio of dogs fed different diets containing different sorghum varieties or rice

[1]Diet Comparison	[2]First Choice	Intake Ratio
BS vs WSA	14	0.362
WSB vs WSA	4*	0.172*
Rice vs WSA	28*	0.657

[1] WSA- white sorghum A, WSB- white sorghum B, BS- burgundy sorghum
[2] First choice was out of 40 total observations
[*] Comparison differs ($P < 0.05$)

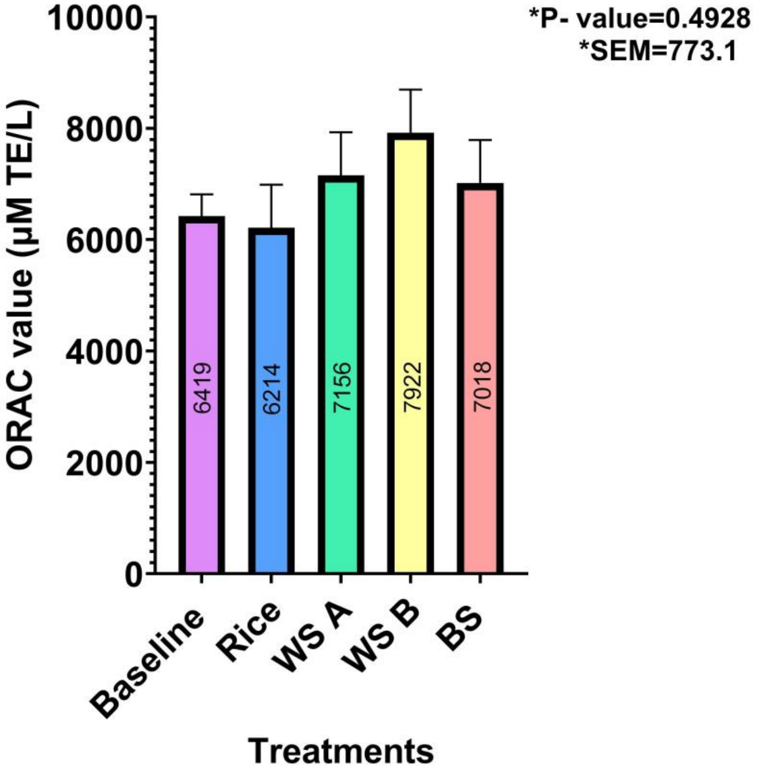

Figure 3-1 Fasted oxygen radical absorbance capacity (ORAC) values in plasma of healthy adult dogs prior to the study (baseline; *not included in statistical analysis) and when fed experimental diets containing either rice, white sorghum A (WSA), white sorghum B (WSB), or burgundy sorghum (BS)

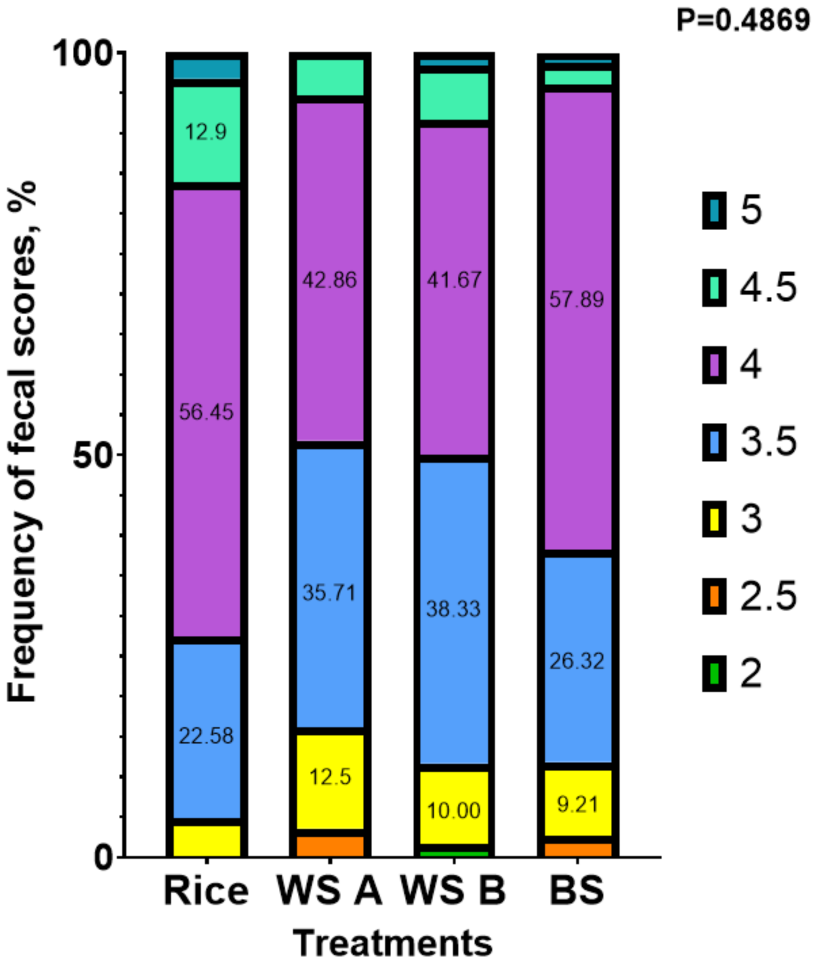

Figure 3-2 Fecal scores by frequency fed experimental diets containing either rice, white sorghum A (WSA), white sorghum B (WSB), or burgundy sorghum (BS) where 1= liquid diarrhea, 1.5 = liquid stool with slight consistency 2= soft, unformed stool 2.5= very moist, some retaining shape, 3= moist, retains shape, 3.5 firm, leaves a slight mark when picked up 4= firm, formed shape, does not leave mark when picked up 4.5= hard, dry stool, cracks when pressure is applied and 5= dry, hard, pellet like, crumbles when pressure is applied

Chapter 4 - Effect of Seed Growth on Nutrient Composition, Phytic Acid, Starch, and *In Vitro* Protein Digestibility: A Preliminary Study

Abstract

Sprouts and microgreens are a novel ingredient for pet food that utilize a seeds ability to change their nutritional composition via germination. Sprouts and microgreens have been reported to increase seed vitamins and minerals as well as phenolic compounds which may provide anti-inflammatory effects. Additionally, germination decreases anti-nutritional factors such as phytic acid. These benefits are attractive qualities in the pet food industry, specifically in the raw food segment as these germinated seeds would be considered unprocessed. Therefore, the objectives of this study were to evaluate the nutritional composition, phytic acid, and *in vitro* protein digestibility of five common cereal grains (corn, millet, sorghum, soybean, and wheat) in different stages of processing (raw, sprouts, and microgreens). To do so, seeds were disinfected with 1% hypochlorite, soaked in water for 16 h to reduce germination time, and randomly placed on tray lined with newspaper. Trays were placed into a commercial baking rack which was in a room with forced air where we attempted to control temperature and humidity. There was a grain by processing effect ($P < 0.05$) in dry matter, ash, fat by hydrolysis, soluble dietary fiber, gross energy, total starch, resistant starch, soluble starch, phytic acid and *in vitro* protein digestibility. Phytic acid was lower ($P < 0.05$) in the microgreens compared to the sprouts and raw treatments for sorghum, millet and wheat. *In vitro* protein digestibility was greatest ($P < 0.05$) in microgreens compared to the raw treatments in sorghum, corn, and millet. Both sprouts and microgreens provide promising nutritional compositions for pet food ingredients and should be further investigated.

Keywords: raw, sprouts, microgreens, germination, pet food

1. Introduction

The pet food industry continually searches for novel ingredients as a means of meeting consumer demands, fostering innovation and staying competitive within the marketplace. Many trends seen in this domain mirror those in the human food industry, as numerous pet owners want to feed their dogs diets similar to their own, with a strong preference for less processed and natural ingredients (Buff et al., 2014). Sprouts and microgreens have gained popularity within the human food industry (Aloo et al., 2021, Bhaswant et al., 2023, Benincasa et al., 2019) and align with emerging trends in the pet food sector, showing promise as potential ingredients in diets formulated for dogs and cats. Sprouts are seeds that are harvested before any greenery has grown but after germination has begun (Benincasa et al., 2019). Microgreens are when the plant (vegetable, grain, herbs) have grown first leaves (Bhaswant et al., 2023). Both can be produced indoors in a hydroponic system with or without soil (Benincasa et al., 2019). In terms of production time, sprouts typically take 1-3 days to germinate, while microgreens can take anywhere from 7-21 days depending on the type of plant and system (light, temperature, humidity; Bhaswant et al., 2023). These ingredients are typically served at high-end restaurants and provide high levels of vitamins, minerals and potential health benefits that many may consider a “superfood.” Both sprouts and microgreens have shown promise to promote health by providing antioxidants that may reduce inflammation, diabetes, obesity, and cardiovascular diseases (Bhaswant et al., 2023, Aloo et al., 2021, Benincasa et al., 2019, Ikram et al., 2021). As the seeds germinate and grow, many qualitative and/or quantitative chemical and nutritional changes occur within starch, protein, minerals, and phenolic acids, improving the seeds’ overall nutritional value (Hübner and Arendt, 2013, Benincasa et al., 2019). Previous studies have evaluated these changes in seeds; however, contradictions on whether nutrients and compounds (starch, protein, phytic acid, phenolic acids) increase, decrease, or stay the same tend to depend on the grain/seed type (Butkute et al., 2019, Sibian et al., 2017, Demir and Bilgicil, 2020, Benincasa et al., 2019). Despite this, it is agreed that microgreens are high in vitamins and minerals, starch is hydrolyzed as the seeds grow, and nutrients are mobilized (Benincasa et al., 2019, Bhaswant et al., 2023). Overall, the germination process (sprouting and microgreens) makes the nutrients more bioavailable and digestible to animals by reducing phytic acid and mobilizing nutrients (Liu et al., 2022).

Sprouted seeds and microgreens may be attractive ingredients to the pet food industry for multiple reasons. First, germination naturally reduces antinutritional factors and enhances nutrient availability making them more digestible for the animal, without the intervention of heat or mechanical energy (Aloo et al., 2021, Benincasa et al., 2019, Bhaswant et al., 2023). Consequently, sprouted seeds and microgreens could be used in raw pet food without compromising its raw status. Due to the lower digestibility of raw starch compared to cooked starch in dogs and cats (Wolter et al., 1998, Schunemann et al., 1989), heat-processed carbohydrate sources are now included in 'raw' diets (Sandri et al., 2019, Sandri et al., 2020, “Carbohydrates in Raw Diets – Canine Nutrition Ingredient Guideline”, 2021). Second, the indoor production that is used for microgreen and sprouts has become more innovative and sustainable compared to outdoor crop production as it allows them to grow in towers without taking up farmland and reduces water use, although, it has a higher input cost (Lone et al., 2024, Stein 2021). Third, extrusion processing, typically used to produce kibbles, can reduce vitamins and affect mineral interactions in a food matrix (Riaz et al., 2009, Gulati et al., 2020), whereas germination increases the their availability(Žilić et al., 2014, Sokrab et al., 2012, Gunathunga et al., 2024, Liu et al., 2022, Ikraam et al., 2021). Corn, wheat, sorghum, millet, and soybeans are grains that are currently used in the pet food industry (ShreeNee, 2023) and their sprouted and (or) microgreens would be a promising ingredient for the minimally processed sector. However, data evaluating the nutritional composition of these germinated products is limited. Most studies that evaluate microgreens tend to choose radish, kale, broccoli, or alfalfa (Di Bella et al., 2020, Baenas et al., 2016, Waterland et al., 2017, Reed et al., 2018, Yadav et al., 2019, Weber, 2017). To our knowledge, few studies have evaluated the nutritional composition of more typical cereal grains as microgreens in addition to sprouts (Niroula et al., 2019, Ebert et al., 2017, Viltres-Portales et al., 2024). We hypothesized that the starch and phytic acid concentrations would decrease and that *in vitro* protein digestibility would increase as the seeds grew. Therefore, the objectives of this preliminary study were to evaluate and compare the nutritional composition, phytic acid content, and *in vitro* protein digestibility of five grains (corn, millet, sorghum, soybean, and wheat) as raw seeds, sprouts, and microgreens.

2. Materials and Methods

2.1 Raw materials and study design

Five common Kansas grains (wheat, sorghum, corn, soybean, millet) were obtained from local elevators from KS. Each grain was evaluated in its raw form and processed to produce sprouted and microgreen seeds, with three replicates for each. Treatments were arranged in a 5 x 3 factorial with main effects of grain (wheat, sorghum, soybean, corn, millet) and processing (raw, sprouted and microgreens) and their interaction evaluated.

2.2 Sprouted and microgreens production

To start, one kg of each replicate from every treatment, including raw cereals, was weighed. Sprouted and microgreen treatments seeds were disinfected with 1% sodium hypochlorite for 30 min then rinsed with distilled water. The sprouted and microgreen seeds were then soaked in water for 16 h to decrease germination time. After the soaking period, each replicate from sprouted and microgreen were randomly assigned to a baking sheet pan placed in a commercial baking rack. A single layer of newsprint to hold moisture for the seeds was placed on each half of the sheet-pans. Seeds were placed randomly on half of a given pan and watered daily to keep them moist. Temperature and humidity were recorded twice daily. There was an attempt to keep temperature and humidity consistent, however, the room relies on forced air heating, which inherently leads to substantial temperature fluctuation. Seeds were considered sprouts when they had a small white shoot emergence. Microgreen seeds were defined as seeds with 1-inch green shoots. When the majority (≥ 75%) of all three replicates met the requirements to be considered sprouted or vegetative seeds, they were sampled and harvested. Unfortunately, during our study there was an issue with mold, which did result in most of the seeds not reaching the microgreen status originally defined. However, given that most seeds had started to grow some greenery and had been germinating for 12 days, it was decided that they had sufficient time to change nutritionally and would still be considered “microgreens” for this preliminary study. The seeds were then dried in a 55°C oven for partial dry matter and stored in glass jars until further analysis.

2.3 Nutrient Analysis

Seeds were ground to 1 mm using a hammer mill (Thomas Wiley Mill, Thomas Scientific LLC, Swedesboro, NJ). Samples were analyzed for moisture (AOAC 930.15), ash (AOAC 942.05), fat by acid hydrolysis (ISO 11085:2008), nitrogen (AOAC 990.03) using a nitrogen analyzer (FP928, LECO Corporation, Saint Joseph, MI) to calculate crude protein (6.25 factor), gross energy (Parr 6200 Calorimeter, Parr Instrument Company, Moline, IL), and insoluble (IDF) and soluble dietary fiber (SDF), with total dietary fiber (TDF) calculated by summing up IDF and SDF (AOAC 991.43). Phytic acid was analyzed using a phytic acid/total phosphorous kit (K-PHYT Megazyme Inc, Wicklow, Ireland). Resistant and soluble starch were analyzed using a resistant starch kit (K-RSTAR Megazyme Inc, Wicklow, Ireland).

2.4 *In Vitro* Protein Digestibility

In vitro protein digestibility was analyzed according to de Godoy et al (2009) with modifications. One gram of each sample was weighed into 50 mL falcon tubes in duplicate. Two additional blanks were created with 1 mL of deionized water. Fifteen mL of 0.1N HCl-pepsin was added to each tube and tubes were placed into a gently shaking water bath for 6 h at 37°C. After 6 h 7.5 mL of 0.5N NaOH was added to each tube to neutralize the reaction and stop hydrolysis of pepsin. Next, a mixture of 4 mg of pancreatin, 7.5 mL of phosphate buffer (pH 8) and 1 mL sodium azide was added to each test tube to initiate pancreatin digestion and prevent microbial growth. The tubes were placed back into the shaking water bath for an additional 18 h at 37°C. After 18 h, 1 mL of trichloroacetic acid was added to each tube to end the reactions and tubes were centrifuged at 20,000xg for 5 min and decanted over Whatman 541 filter paper. The supernatant was discarded, and 30 mL of deionized water was added to each tube. Tubes were vortexed, re-centrifuged at 20,000 x g for 5 min. The process was repeated 3 times or until the supernatant was clear. Then a spatula and deionized water were used to decant all precipitant onto the filter paper. Filter paper was then dried overnight at 105°C and weighed the next morning. Crude protein by nitrogen (AOAC 990.03) was then analyzed using a nitrogen analyzer (FP928 LECO Corporation, Saint Joseph, MI). Protein digestibility was calculated using the equation below.

$$CP\ Digestibiliity = \frac{g\ sample\ CP - g\ residue\ CP}{g\ sample\ CP} * 100$$

2.5 Statistical Analysis

Nutrient composition and *in vitro* protein digestibility were analyzed as a completely randomized design. The main effect of grain, processing and their interaction were evaluated in a fixed-effect statistical model using the GLIMMIX procedure in SAS (v. 9.4, SAS Institute, Inc, Cary, NC). Means were separated using Fisher's LSD and a probability of $P < 0.05$ was accepted as significant.

3. Results

3.1 Dry Matter

A grain by processing type effect ($P < 0.0001$) was observed for dry matter content (Table 4-1). Wherein, dry matter content of raw corn (94 %) was greater compared to sprouts (78%; $P = 0.0002$) and microgreen (85%; $P = 0.0326$) treatments. For millet, dry matter content was greater ($P = 0.0021$) in the raw (92%) compared to microgreens (79%), which was greater ($P < 0.0001$) than the sprout (55%) treatment. Dry matter content was similar in the sorghum raw (90%) and microgreen (92%; $P = 0.5962$) and the soybean raw (92%) and microgreen (88%; $P = 0.2185$) treatments, which were both greater compared to the sorghum sprouts (65%; $P < 0.0001$) and soybean sprouts (47%; $P < 0.0001$) treatment. Finally, dry matter content was greater ($P < 0.0001$) in raw wheat (94%) followed by sprout (61%; $P<0.0001$) and microgreen (34%) treatments.

3.2 Ash

A grain by processing type effect ($P = 0.0018$) was observed for ash content. The ash content for raw corn (1.8%) and wheat (2.2%) was greater compared to the sprouted corn (1.1%;

$P = 0.0071$) and wheat (1.7%; $P = 0.0392$) treatments. Corn microgreens (1.5% ash) were similar to raw corn ($P = 0.2346$) and sprouts ($P = 0.1041$). Additionally, wheat microgreens (2.1% ash) were similar to raw wheat ($P = 0.5675$) and sprouted wheat ($P = 0.1251$). For millet, the raw seeds had the greatest ash content (3.7%) compared to the sprouts (2.6%; $P = 0.0001$) and microgreens 2.2%; ($P < 0.0001$). Sorghum microgreens had the lowest ash content (2.9%) compared to the raw (4.0%; $P = 0.0002$) and sprouted (3.5%; $P = 0.0338$) treatments. No differences ($P > 0.05$) were observed in soybean (4.7%).

3.3 Crude Protein

An effect of grain ($P < 0.0001$), but no effect of processing and grain by processing interaction were observed for crude protein ($P = 0.3106$). Wherein, soybean had the greatest ($P < 0.05$) protein (32.5-35.6%) content which was almost twice the amount of protein as the other grains. Millet had lower ($P < 0.05$) protein content (17.0-19.0%) than soybean but greater ($P < 0.05$) than wheat (13.7-16.0%). Sorghum had a lower ($P < 0.05$) protein content (10.3-10.7%) than wheat. Corn had the lowest ($P < 0.05$) protein content among all grain types (8.6-9.3%).

3.4 Fat by Acid Hydrolysis

A grain by processing type effect was observed ($P = 0.0003$) for fat by hydrolysis. Raw millet had a greater (14.0%; $P = 0.0024$) fat content than the sprouted (11.6%) treatment, which had a greater ($P = 0.0135$) fat content than the microgreens (9.7%). For sorghum, the microgreen treatment had lower fat content (5.9%) than the raw (8.0%; $P = 0.0074$) and sprouted (8.0%) treatments ($P = 0.0066$), which were similar ($P = 0.9632$). Raw soybean had a lower fat content (24.6%) compared to sprouted soybean (27.0%; $P = 0.0021$) and soybean microgreens (26.4%; $P = 0.0181$), which were similar ($P = 0.3957$). No differences ($P > 0.05$) were observed in corn (6.5-7.2%) or wheat (2.8-3.7%) treatments.

3.5 Total Dietary Fiber

An effect of grain ($P<0.0001$), but no effect of processing and grain by processing interaction were observed for total dietary fiber ($P = 0.8409$). Soybean had the greatest ($P < 0.05$) total dietary fiber (TDF) content among grain types (29-34%). Corn had the least ($P < 0.05$) TDF content among grain types (18-21%), while millet (22-27%), sorghum (21-23%), and wheat (20-27%) were intermediate ($P < 0.05$).

3.6 Insoluble Dietary Fiber

An effect of grain ($P<0.0001$), but no effect of processing and grain by processing interaction were observed for insoluble dietary fiber. Soybean had the greatest ($P < 0.05$) insoluble dietary fiber (IDF) content among all grain types (29-32%). Wheat had lower ($P < 0.05$) IDF content (20-26%) than soybean but greater ($P < 0.05$) IDF content than sorghum and corn. Millet had similar ($P > 0.05$) to wheat (20-24%). Corn had the lowest ($P < 0.05$) IDF content among all grain types (17-21%). Sorghum had similar ($P > 0.05$) IDF content to millet and corn (29-22%).

3.7 Soluble Dietary Fiber

A grain by processing type interaction was observed ($P = 0.0366$) in soluble dietary fiber. Wherein, raw millet had the greatest ($P = 0.0275$) content of SDF (3.1%) compared to the sprouted treatment (1.9%). Millet microgreens were similar to both raw ($P = 0.1029$) and sprouted millet (2.2%; $P = 0.5305$). Conversely, raw soybean had lower SDF (0.3%) compared to soybean microgreens (1.9%; $P = 0.0086$) and sprouts (1.5%; $P=0.0441$), which were similar ($P=0.4835$). No differences ($P > 0.05$) were observed in corn (0-1%), sorghum (1.3-2.3%), or wheat (0.3-1.0%) treatments.

3.8 Gross Energy

A grain by processing type interaction was observed (P=0.0263) in gross energy. Sprouted sorghum had greater gross energy (4.8 kcal/g) than raw sorghum (4.4 kcal/g; P = 0.0025) and sorghum microgreens (4.5 kcal/g; P = 0.0149), which were similar in gross energy (P = 0.4822). No differences (P > 0.05) in any other grains were observed. Corn had an average of 4.4 kcal/g, millet an average of 4.8 kcal/g, soybean an average of 5.8 kcal/g, and wheat and average of 4.5 kcal/g.

3.9 Total Starch

A grain by processing type interaction was observed (P < 0.0001) for total starch. Raw sorghum had the greatest (P = 0.0431) total starch content (57.4%) compared to the sprouted treatment (52.6%). Sorghum microgreens (56.6%) were similar to both sprouts (P = 0.0870) and raw sorghum (P = 0.7341). For millet, microgreens had the greatest total starch content (49.3%) compared to raw (40.1%; P = 0.0003) and sprouted millet treatments (41.5%; P = 0.0017), which were similar (P = 0.5455). Conversely, wheat microgreens had the least (P<0.0001) total starch (37.6%) content compared to its raw (51.3%) and sprouted (51.1%) counterparts, which were also similar (P = 0.9423). No differences (P > 0.05) were observed in corn (59.7-64.2%) or soybean treatments (52.6-57.4%).

3.10 Soluble Starch

A grain by processing type interaction was observed (P < 0.0001) for soluble starch herein, raw corn had lower soluble starch (58.9%) compared to corn sprouts (63.6%; P = 0.0445) and microgreens (63.5%; P = 0.0496), which were similar (P=0.9599). For millet, microgreens had the greatest soluble starch content (49.1%) compared to the raw (38.9%; P = 0.0001) and sprouted millet (63.6%; P = 0.0017) treatments. Conversely, wheat microgreens had the least soluble starch content (37.6%) compared to its raw (50.8%; P < 0.0001) and sprouted (50.9%; P < 0.0001) treatments, which were similar (P = 0.9694). No differences (P > 0.05) were observed in sorghum (51.7-56.0%) or soybean treatments (3.9-4.2%).

3.11 Resistant Starch

A grain by processing type interaction was observed (P < 0.0001) for resistant starch. Resistant starch content was the greatest in the raw corn (0.81%; P = 0.0026), millet (1.21%; P < 0.0001), and sorghum (1.69%; P < 0.0001) treatments compared to the sprouted corn (0.56%), millet (0.49%), and sorghum (0.93%) treatments, which was greater than the corn (0.38%; P = 0.0301), millet (0.24%; P = 0.0034), and sorghum (0.60%; P = 0.0002) microgreens. For wheat, resistant starch content was greatest in the raw treatment (0.49%) compared to the sprouted (0.23%; P = 0.0024) and microgreen (0.21%; P = 0.0013) treatments, which were similar (P = 0.8185). No differences (P > 0.05) were observed in soybean treatments (0.03-0.05%).

3.12 Phytic Acid

A grain by processing type interaction was observed (P<0.0001) for phytic acid content. Phytic acid was greatest in the raw millet (1.6%; P = 0.0001) and wheat treatment (1.2%; P = 0.0041) compared to the millet sprouts (1.1%) and wheat sprouts (0.8%), which was greater than the millet (0.7%; P = 0.0004) and wheat (0.6%; P = 0.0474) microgreen treatments. Sorghum microgreen treatment also exhibited the lowest phytic acid content (1.0%) compared to the sprouted (1.5%; P < 0.0001) and raw treatments (1.6%; P < 0.0001), which were similar (P = 0.3095). No differences (P < 0.05) were observed in corn (0.9%) or soybean treatments (1.1%).

3.13 *In Vitro* Protein Digestibility

A grain by processing type interaction was observed (P = 0.0099) for *in vitro* protein digestibility. Wherein, corn microgreens had the greatest (94.8%; P = 0.0118) IVPD compared to the raw treatment (91.9%). Millet microgreens had the greatest (86.6%; P = 0.0358) IVPD compared to the sprouted treatment (84.2%). For sorghum, the raw treatment had lower IVPD (80.7%) compared to the sprouts (83.1%; P = 0.0345) and microgreen (83.8%; P = 0.0081) treatments, which were similar (P = 0.5376). Soybean sprouts had greater IVPD (95.8%) than the microgreen (91.9%; P = 0.0013) or raw treatments (92.9%; P = 0.0114). No differences (P > 0.05) were observed in wheat treatments (90.7-92.0%).

4. Discussion

The objective of this study was to evaluate microgreens and sprouts from five different cereal grains to select one to undergo high production significant for an animal study. However, we encountered difficulties with growing seeds to the microgreen stage with our growing apparatus. Mold was a major issue and led to the decision to harvest seeds early, which then resulted in the need to alter our original definition of microgreens for this preliminary study. Because it is believed we gave ample time for the seeds to grow and green shoots to emerge on all seeds we still considered them microgreens as such, however, only wheat was truly able to produce microgreens with over 2.54 cm of greenery (Figure 4-4 to 4-6). Dry matter for all microgreens were expected to be lower than raw or sprouts, similar to wheat, however, because it was decided to harvest seeds early due to mold, the soybean, corn, sorghum, and millet microgreens were not watered the morning of harvest in order to avoid extra mold growth. Therefore, the dry matter was greater in the microgreen treatments of those grains. Germination causes many changes in a seed’s nutritional and chemical composition. Thus, it was expected that we would observe changes in the nutritional content of raw compared to sprouts and microgreens. Ash was the highest in the raw seeds for corn, millet, wheat, and sorghum compared to their sprout treatments. However, no changes were observed for soybean. Sibian et al. (2017) did not observe changes in the ash content of wheat, triticale, or brown rice sprouts compared to their raw seeds (0.1-0.2% difference). Conversely, Butkute et al. (2018) reported a greater average ash content in seven different legume microgreens when compared to their raw seeds (1-3% increase). Furthermore, Butkute et al. (2018) analyzed specific minerals that are of interest in legume sprouts and microgreens. Magnesium, potassium, zinc, and phosphorous were highest in the microgreen treatments compared to sprouted and raw seeds, whereas the raw seeds had the greatest calcium levels (Butkute et al., 2018). Thakur et al. (2021) also reported greater mineral concentrations of calcium, iron, zinc, and manganese in amaranth, buckwheat, and quinoa as the sprouted seeds continued to grow. We did not analyze specific mineral concentration as this was more of a preliminary study, however, future studies should consider evaluating the effect of germination on specific mineral concentration to provide a more comprehensive nutritional composition due to this process. Crude protein was not different among processing type in this study; however, it did differ among the grains tested, which was expected because each raw grain is known to have different protein percentages. Unlike our study, Butkute et al. (2018) observed that microgreens increased 15% in crude protein in red clover, alfalfa (regular and black medic) and sainfoin compared to the raw seeds and 8% compared to the sprouted seeds. Sibian et al. (2017) and Thakur et al. (2021) evaluated crude protein on germinated wheat, triticale, brown rice, amaranth, buckwheat, and quinoa and reported, sprouts had a greater protein content compared to the raw seeds (16-28% increase for wheat, brown rice, and triticale at 94 h germination, 1-3% increase for amaranth, buckwheat and quinoa at 72 h germination). However, in germinated brown rice flour, no differences in crude protein were reported (Xu et al., 2011). Furthermore, an increase in total free amino acids was observed in the germinated brown rice flour (Xu et al., 2011). Mohan et al. (2010) reported that proteins that contain lower molecular weights (≤35 kDA) remain unchanged during germination. Therefore, the difference may be prevalent at the amino acid level and future studies should evaluate free amino acids and protein molecular weight in addition to crude protein. In our study, IVPD was the greatest for microgreens in corn, millet, and sorghum. Moreover, soybean sprouts had greater IVPD than the raw seeds. Previous studies also found that sprouting seeds resulted in greater IVPD in sorghum (Nour et al., 2010), finger millet (Hejazi and Orsat, 2016), foxtail millet (Sharma and Gujral, 2020), barnyard millet (Sharma and Gujral, 2020), amaranth (Hejazi et al., 2016), chickpeas (El-Adawy, 2002), lentils and cowpeas (Ghavidel and Prakash, 2007) compared to their raw seeds. Additionally, high phytate/phytic acid levels have been negatively correlated with protein digestibility (Sharma and Gujral 2020, Singh et al., 1991, Chitra et al., 1995). With the observed reduction in phytic acid levels in sorghum and millet, it's plausible that the rise in IVPD could be associated with this change. However, this hypothesis is not entirely supported in our study as corn microgreens had greater digestibility, but the phytic acid content was not affected by germination. This could potentially stem from changes at the amino acid level rather than differences in phytic acid content. For example, in a review by Bera et al. (2023), total free amino acids and IVPD increase after germination in different types of legumes and grains (chickpeas, lentils, sorghum, soybean). In the current study, most of the raw cereal grains matched up with the protein contents listed on Feed Tables (“*Feed profile | Tables of composition and nutritional values of feed materials INRA CIRAD AFZ.*”, 2021). Raw millet was the most different in crude protein content as it was listed as 12.5% (dry matter) and we analyzed it at 19.0% (dry matter). It is believed that seeds convert fat and oils into sugar via beta oxidation and glyoxylate cycles during germination (Benincasa et al., 2019). Therefore, it was expected that fat would be lower in the microgreen treatment. Sorghum and millet had the lowest fat content in their microgreen treatments and the greatest fat content in their raw treatments. This confirms previous studies that also found that germination reduces the fat content in cereal/pseudocereal grains and seeds (Butkute et al., 2018, Thakur et al., 2021, Chinma et al., 2009). Conversely, soybean had the opposite effect. Soybean microgreens had the highest fat content while its raw seeds had the lowest. Sibian et al. (2017) also reported that wheat and brown rice had a greater fat content in their sprouted treatment compared to their raw form. Furthermore, Durairajan et al. (2023) reported that a sprouted millet treatment had a lower crude fat content than for microgreens. Sibian et al. (2017) reported that the oil absorption capacity was increased after germination, and it was positively correlated with protein quality and was likely the cause of increased fat rather than the expected decrease. This may help explain the highest fat content in soybean microgreens. Moreover, Kai et al. (2022) found that soybean protein isolate had a high oil holding capacity and was advantageous for high oil foods. The only difference observed in gross energy was for sorghum sprouts. Keyata et al. (2021) found that there were no differences in gross energy observed between raw and germinated sorghum grain, however, the soaked seeds had a greater energy content than both raw and germinated.

Concentration and the composition of dietary fiber has been reported to increase during germination, however, the changes are inconsistent and often depend on germination conditions and grain type (Benitez et al., 2013, Benincasa et al., 2019). Total dietary fiber and IDF were not affected by processing but differed by grain type. Previous studies found that both sprouts and microgreens had greater dietary fiber content compared to their raw treatments (Sibian et al. 2017, Butkute et al. 2018). Insoluble dietary fiber ranged from 18.6-31.2% in the cereal grains used in this study (corn being the lowest and soybean the highest). Cereal grains can contain beneficial compounds such as IDF that may provide health benefits to humans or animals, such as satiety and energy dilution; both of which can assist in weight control (Hamedani et al., 2009, Lattimer et al., 2010). The IDF fraction may also contain small amounts of polyphenols, which in sufficient quantities may benefit gut health (Timm et al., 2023). Unfortunately, in the current study, polyphenols or total phenolic content were not analyzed. Something which should be considered for the next study. On the other hand, there are published studies that evaluate the impact of germination on antioxidants, polyphenols and total phenolics (Donkor et al., 2012, Durairajan et al., 2023, Ikram et al., 2021, Galieni et al., 2020, Wojdylo et al., 2020, Li et al., 2022) and generally resulted in greater polyphenols, total phenolic content and (or) antioxidant activity. This would place germination as a feasible method to improve potential functional ingredients that promote overall health.

There was a processing effect on SDF within grain type. Millet had the greatest SDF content in the raw compared to the sprouted treatment, while soybean had the greatest SDF in sprouts and microgreens compared to raw. However, all treatments had low SDF content (≤ 3%). Koehler et al. (2007) reported that wheat SDF content was greater after 7 days of germination compared to raw seeds. However, a previous study observed similar SDF contents between raw and germinated (72h) brown rice (Ohtsubo et al., 2005). Temperature may have an effect on the nutritional changes resulting from germination. For example, Koheler et al. (2007) found differences in SDF in wheat germination at different temperatures. Wherein, at 15°C and 20°C, there was a threefold increase in SDF and 50% decrease in IDF. In the current study, we tried to control the temperature, however, due to forced air heating-cooling system temperatures wavered 10° (19-29°C) throughout the duration of the experiment.

One of the most significant nutritional changes that results from germination is starch content and availability (Zeeman et al., 2010, Benincasa et al., 2019). In the current study, *in* *vitro* starch and organic matter digestibility were not evaluated. Nevertheless, findings revealed a decrease in total starch for sorghum and wheat, and an increase for millet. Soluble starch decreased in wheat but increased in corn and millet, while resistant starch decreased across all grain types except soybeans. Previous studies documented a decrease in total starch in raw seeds compared to sprouts (Sweica et al. 2013, Sibian et al., 2017, Farzaneh et al., 2017). This reduction is mostly attributed to the hydrolysis of starch by alpha-amylase during germination. Therefore, it was expected that total starch would be lower in the sprouted and microgreen treatments compared to the raw seeds. While this hypothesis was true for wheat, it deviated for millet and sorghum. Millet microgreens had the highest total starch and sorghum microgreens had a similar total starch content to their raw seeds. Millet and corn had greater levels of soluble starch in their microgreens and sprouted treatments compared to their raw seeds. This finding corroborates with Durairajan et al. (2023) who also reported microgreens containing greater levels of soluble starch than their sprouted treatment. Conversely, the opposite effect was observed for wheat, where microgreens had the lowest amount of soluble starch. Sweica et al. (2013) also reported lower levels of bioavailable starch in sprouts compared to soaked and raw seeds. Additionally, all seeds except soybean had lower resistant starch in the microgreen treatment compared to the raw seeds. Sweica et al. (2013) reported a lower resistant starch content in the sprouts compared to soaked or raw seeds. In a recent study, the longer Bambara groundnut were germinated, the lower the resistant starch (Chinma et al., 2023). This affirms the results observed for millet, corn, and sorghum in the current study. Conversely, when wheat bread was enriched with sprouted wheat flour, greater concentrations of resistant starch were observed with the inclusion of wheat flour (5-20% or full replacement) compared to the control bread containing wheat flour. The authors explained that the sprouted wheat flour was high in resistant starch, which may be due to tannins interacting with flour (Sweica et al., 2017 and Mkandawire et al., 2013). This was not seen in our study since millet and red sorghum are reported to be tannin-free in the United States (reference).

A major digestibility challenge with raw seeds for non-ruminant species is the phytic acid content. Phytic acid lowers the availability of minerals and nutrients by binding and making them unavailable to the animal (Feizollahi et al., 2021). According to Wu et al. (2009), phytic acid can make up 60-90% of a cereal grain's total phosphorous levels. Germination has been reported to decrease phytic acid (Gupta et al., 2013, Feizollahi et al., 2021). In the current study, it was found that among the grains, except soy and corn, phytic acid decreased as the seed grew. This is consistent with the results of Azeke et al. (2011) who evaluated the effect of germination time on phytic acid content in rice, corn, millet, sorghum, and wheat decreased in the concentration of phytic acid in all grains as the days of germination increased. Furthermore, previous studies have reported lower concentrations of phytic acid after germination (Maldonado-Avarado et al., 2023), or decreasing levels of phytic acid the longer germination occurred in quinoa, amaranth, wheat, and rice (Pakfetrat et al.,2019; Thakur et al.,202; Wu et al., 2022). Further evaluation may be necessary for corn and soy to determine if there is truly a reduction in phytic acid content with advancing germination.

5. Conclusion

This preliminary study allowed for the investigation of nutritional changes occurring during the germination of various cereal grains. However, when planning for larger-scale production, a more efficient growing apparatus should be considered. Germination resulted in grain by processing interactions for dry matter, ash, fat by acid hydrolysis, SDF, gross energy, total starch, resistant starch, soluble starch, phytic acid and IVPD, indicating that chemical and nutritional changes resulting from germination should be evaluated individually within each grain and not generalized as a uniform outcome among different raw materials. Similarly, changes in total and soluble starch contents as well as SDF differed by grain. The lower phytic acid content and greater IVPD observed in sprouted and microgreens from cereal grains compared to their raw seeds is a promising preliminary result to consider for the utilization of germinated seeds in dog and cat food that are intended for raw consumption. Further studies should investigate the safety, palatability, and digestibility of incorporating sprouts and microgreens derived from cereal grains into dog and cat diets to effectively introduce this approach for preparation and use in modern pet foods.

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Table 4-1 Effect of grain and processing type (raw, sprout, microgreen) on nutritional composition, phytic acid content, and *in vitro* protein digestibility (dry matter basis)

						P-Values		
Item, %	Corn	Millet	Sorghum	Soybean	Wheat	Grain	Process	Grain x Process
Dry Matter						<0.0001	<0.0001	**<0.0001**
Raw	93.9^{a}	91.8^{a}	90.1^{a}	92.4^{a}	93.9^{a}			
Sprout	77.7^{b}	54.7^{c}	65.1^{b}	46.6^{b}	61.0^{b}			
Microgreen	85.3^{b}	78.8^{b}	92.2^{a}	87.5^{a}	33.9^{c}			
Ash						<0.0001	<0.0001	**0.0018**
Raw	1.8^{a}	3.7^{a}	4.0^{a}	4.7	2.2^{a}			
Sprout	1.1^{b}	2.6^{b}	3.5^{a}	4.6	1.7^{b}			
Microgreens	1.5^{ab}	2.2^{b}	2.9^{b}	4.7	2.1^{ab}			
Crude Protein						**<0.0001**	0.3106	0.0574
Raw	9.3	19.1	10.4	32.5	14.4			
Sprout	8.6	17.0	10.7	35.7	13.7			
Microgreen	9.3	17.8	10.3	35.6	16.0			
Fat by acid hydrolysis						<.0001	0.0009	**0.0003**
Raw	7.2	14.0^{a}	8.0^{a}	24.6^{b}	3.7			
Sprout	6.9	11.6^{b}	8.0^{a}	27.0^{a}	3.5			
Microgreens	6.5	9.7^{c}	5.9^{b}	26.4^{a}	2.8			

Total dietary fiber						**<0.0001**	0.8409	0.0524
Raw	21.0	27.1	22.8	29.3	24.1			
Sprout	17.9	26.1	22.5	33.8	20.4			
Microgreen	18.1	21.8	21.2	34.1	27.4			
Insoluble dietary fiber						**<0.0001**	0.6692	0.0665
Raw	20.7	24.1	21.5	28.9	23.8			
Sprout	16.9	24.3	20.8	32.4	19.8			
Microgreen	18.1	19.6	18.9	32.2	26.4			
Soluble dietary fiber						<0.0001	0.2949	**0.0366**
Raw	0.3	3.1^{a}	1.3	0.3^{b}	0.3			
Sprout	1.0	1.9^{b}	1.7	1.5^{a}	0.6			
Microgreen	0.0	2.2^{ab}	2.3	1.9^{a}	1.0			
Gross energy						<0.0001	0.8891	**0.0263**
Raw	4.5	4.9	4.4^{b}	5.7	4.4			
Sprouts	4.3	4.7	4.8^{a}	5.8	4.4			
Microgreens	4.4	4.8	4.5^{b}	5.8	4.6			
Total starch						<0.0001	0.8997	**<0.0001**
Raw	59.7	40.1^{b}	57.4^{a}	4.2	51.3^{a}			
Sprout	64.2	41.5^{b}	52.6^{b}	4.3	51.1^{a}			
Microgreen	63.9	49.3^{a}	56.6^{ab}	3.9	37.6^{b}			

Soluble starch						<0.0001	0.0207	**<0.0001**
Raw	58.9^{b}	38.9^{b}	55.7	4.2	50.8^{a}			
Sprout	63.6^{a}	41.0^{b}	51.7	4.2	50.9^{a}			
Microgreen	63.5^{a}	49.1^{a}	56.0	3.9	37.4^{b}			
Resistant starch						<0.0001	<0.0001	**<0.0001**
Raw	0.81^{a}	1.21^{a}	1.69^{a}	0.03	0.49^{a}			
Sprout	0.56^{b}	0.49^{b}	0.93^{b}	0.05	0.23^{b}			
Microgreen	0.38^{c}	0.24^{c}	0.60^{c}	0.05	0.21^{b}			
Phytic acid						<0.0001	<0.0001	**<0.0001**
Raw	0.9	1.6^{a}	1.6^{a}	1.1	1.2^{a}			
Sprout	0.9	1.1^{b}	1.5^{a}	1.1	0.8^{b}			
Microgreen	0.9	0.7^{c}	1.0^{b}	1.0	0.6^{c}			
In vitro protein digestibility						<0.001	0.0083	**0.0099**
Raw	91.9^{b}	85.1^{ab}	80.7^{b}	92.9^{b}	90.7			
Sprout	92.6^{ab}	84.2^{b}	83.1^{a}	95.8^{a}	91.2			
Microgreen	94.8^{a}	86.6^{a}	83.8^{a}	91.9^{b}	92.0			

^{abc} Means within a column with unlike superscripts differ (P<0.05)

Table 4-2 Temperature and humidity of each day of growth with averages and standard deviations

Growth Day	Temperature (°C)	Humidity (%)
1	19.8	16
2	25.3	17
3	25.6	25
4	21.9	16
5	25.2	16
6	26.1	19
7	22.2	26
8	24.2	22
9	28.3	19
10	29.4	57
11	27.5	29
12	25.0	19
Average	25.0	23.4
Standard Deviation	2.8	11.4

Figure 4-1 Raw seeds with soybean (top left), wheat (top right), sorghum (bottom left), millet (bottom middle), and corn (bottom right).

Figure 4-2 Commercial baking rack with baking pans that seeds were placed on to germinate

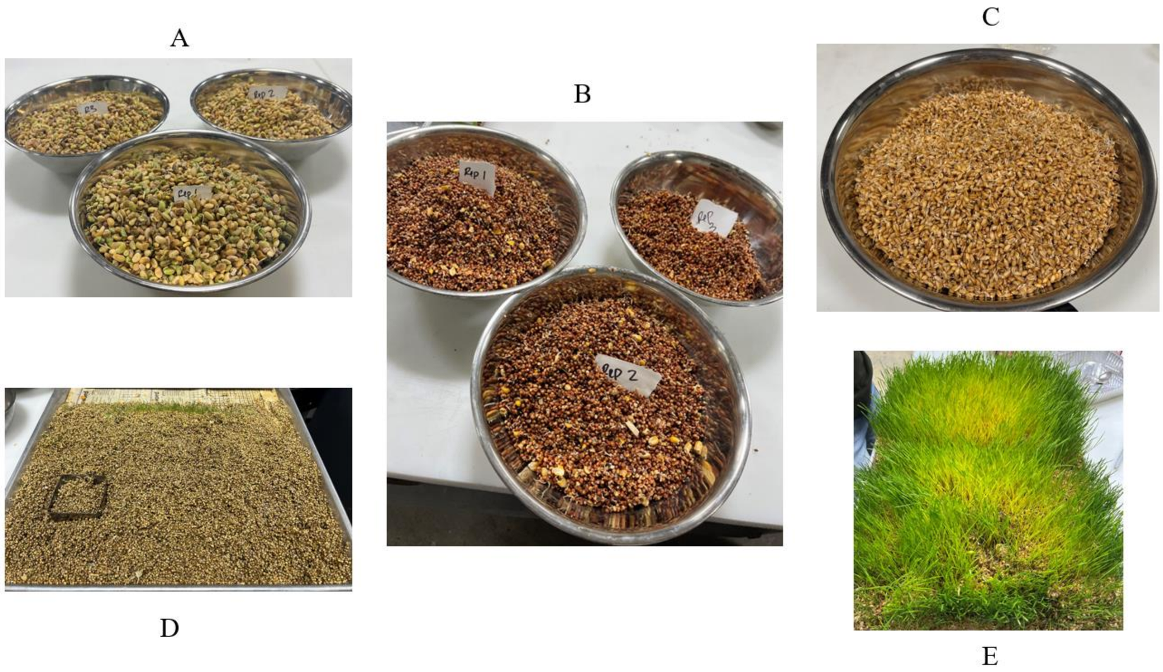

Figure 4-3 A) sprouted soybeans B) sprouted sorghum C) sprouted wheat D) millet microgreens at harvest with some greenery and E) wheat microgreens at harvest

Chapter 5 - Sprout and Microgreen Process Development for Production of Sufficient Volume to Support a Pet Food and Feeding Evaluation Food

1. Introduction

Following promising findings from the preliminary study (Chapter 4), our focus shifted to a single grain, sorghum, chosen due to its previous evaluations in our laboratory's research and its potential health benefits for companion animals. As noted in the preliminary study, the growing apparatus was a limiting factor for seed production due to mold growth. Furthermore, there was a need to cultivate sufficient sprouts and microgreens to produce food for a processing study and for nutritional acceptability evaluation by companion animals. Therefore, the next phase focused on optimizing the process protocol to generate sufficient quality and quantity of microgreens and sprouts to support experimental diet production.

2. Base Growing Substrate Selection

A commercial microgreen tower was constructed and used for the growing of seeds in a university laboratory (Throckmorton Hall, Manhattan, Kansas). The tower was comprised of 16 trays, with 6 lights allocated for every 4 trays, two waterers per tray, and an aerator. Four different base growing substrates were evaluated with seeds at a low density to determine their efficacy. Given the relative ease of achieving sprouts in the preliminary study, subsequent investigations primarily focused on microgreens. The project aimed to obtain seeds fully intact, including the tap root and seed with at least 2.5 cm of green leaf shoot. Newspaper, burlap, and two different cotton substrates (with only color variation, later determined to be similar in content) were tested (Figure 5-2, 5-3, 5-4 and 5-5). Following approximately 10 days of growth, the seeds were harvested. Notably, seeds on the newspaper substrate did not penetrate the newspaper remaining on the surface (Figure 5-5), which resulted in diminished growth compared seeds sprouted on other substrates (Figure 5-6). Additionally, water accumulation on top of the newspaper fostered mold growth. Harvesting from the burlap substrate proved challenging as the roots hooked to the underside, requiring cutting for removal. Therefore, we concluded that the cotton substrates offered the most favorable conditions for our experiment. The seeds' initial low density during this test led to the next step, which was to increase the seed density and a determination of the optimal growing density to maximize yield for production.

3. Density Optimization

To increase the density, the initial evaluation began with 0.45 kg, and 0.68 kg at 117.5 cm length in duplicate. Both densities were evenly distributed across two trays to mitigate potential variations in water flow rates. By day 7, the 0.68 kg density was ready for harvest while the 0.45 kg density took an extra day. The 0.68 kg density produced an average of 582g (dry matter) microgreens, whereas the 0.45 kg density only produced 361 g (dry matter) of microgreens. No issues of ungerminated seeds with the 0.68 kg density were noted. The next step was to test if the density could be increased to 1.5 kg versus the previously successful 0.68 kg density. During this test 0.68 kg produced approximately 401 g (dry matter) and 1.5 kg produced 815 g (dry matter). However, it was observed that a substantial portion of the seeds remained unsprouted and hard, with most of the growth being root rather than green (Figure 5-7, 5-8, and 5-9). Therefore, we concluded that 0.68 kg was the optimal density to achieve the desired growth for our specific requirements under these conditions.

4. Mold Growth

Throughout our testing, mold growth has been consistently observed (Figure 5-10). Therefore, we decided to conduct a test wherein 0.68 kg of sorghum was cleaned with a 1% NaClO- (bleach) prior to placing it on the substate to assess its potential to reduce mold growth. In addition to cleaning, the substrate was changed to burlap, as it was observed that the cotton retained excessive water, potentially contributing to the mold growth. However, as the seeds grew there was no discernable difference in mold growth (Figure 5-11) with the change in growing substrate. Interestingly, the tray without the disinfectant treatment produced more dry microgreens (1130 g vs 918 g). Since molds can produce mycotoxins that are harmful to dogs/cats and other mammals' evaluation was necessary to assure food safety. Selecting from the moldiest portion a subsample was evaluated for alfatoxins levels and determined to be 7.7 ppb, while fumonisin was 0.47 ppm. Both were well below the safety thresholds for consumption (National Grain and Feed Association, 2011). However, the presence of mold remains undesirable. Therefore, the microgreen tower was disassembled with each component disinfected using a diluted bleach solution, followed by rinsing with water, before being reassembled. During this process, it was discovered that the aerator had not been functioning properly prior to this process, likely contributing to the mold issue. The aerator was reconstructed, and the lights were reconnected. Another test batch was produced with 0.68 kg of sorghum per 2,760 cm^2 (117.5 cm length x 23.5 cm width) and production significantly improved with notable reduction in mold to near elimination. At this stage one additional test was conducted to confirm the 7-day timing for microgreens and 2-day timing for the sprouts, both of which yielded successful outcomes (Figure 5-12). Based on these results, we determined this protocol was effective for the growth of sorghum sprouts and microgreens for experimental diet production and evaluation by companion animals, specifically cats.

5. History of Sprouting/Malting

Sprouting grains, also known as malting, has been a technique used for centuries. Briggs (1998) reported that malting grains to produce beer has been practiced for at least 6,000 years. Furthermore, Singer et al. (1954) reported that malting was mentioned in some of the earliest written records. Moreover, Egyptian tomb-paintings depict the beer-making process (Singer et al., 1954) which included malting grains. Throughout the centuries the methods of sprouting/malting have changed slightly. For example, for small batches, grain was steeped in sacks in streams and then drained or by soaking in wooden troughs (Nordland, 1969). However, starting in the 17th century, floor-malting became traditional for a large quantity of malt (Briggs, 1998). Furthermore, in the 19th century large breweries were formed and industrialization of malting occurred and became popular around the world (Briggs, 1998). Moreover, the use of sprouted grains for enhancement of nutritional composition has been studied for the last few decades (Baranwal, 2017, Ikram et al., 2021). Therefore, the idea of malting or sprouting to unlock the nutrients in grains is not new. However, it has not been utilized in pet foods to our knowledge.

6. Food safety of sprouts and microgreens

Sprouted grains are a great source of nutrition as they have mobilized fat, increased protein digestibility and reduced phytic acid (Chapter 4). However, they are a cause of concern due to microbial growth. Sprouts are typically grown in a warm, humid environment that is also favorable to mold growth (Turner et al., 2020). Furthermore, Miyarhira and Antunes (2021) reported that 14,739 foodborne illness outbreaks were related to sprouts between 1988 and 2020.

These outbreaks were due to *Salmonella spp.* and *Escherichia coli* (Miyarhira and Antunes, 2021) species. It has also been reported that sprouts may be more susceptible to pathogens and mold growth compared to microgreens because they are usually soaked in water in troughs without light in very humid environments (Turner et al., 2020). Microgreens are typically rooted in a substrate, exposed to air and light which allows for evaporation and reduces humidity (Turner et al., 2020). In our system we had both sprouts and microgreens growing using the microgreen system which allowed evaporation of water and reduced humidity. Furthermore, the room the microgreen tower was in has cooling pads on the wall which kept the temperature constant and prevented it from getting too warm and creating an environment for mold. Chemical (chlorine, hydrogen peroxide, calcium hydroxide, calcinated calcium), biological (lactic acid bacteria, bacteriophage), and physical methods (high pressure, heat, irradiation, UV light) have been researched to reduce pathogens and mold in sprouts and may be necessary if these are an issue in an operation (Miyahira and Antunes, 2021, Sikin et al., 2013).

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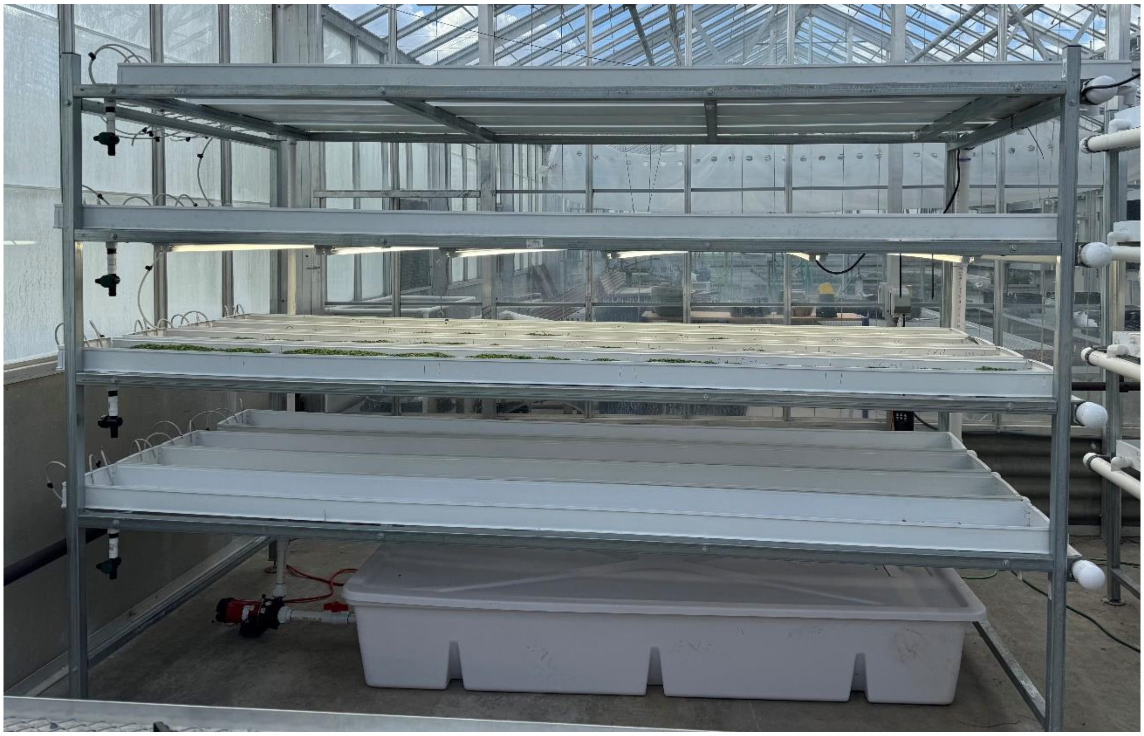

Figure 5-1 Commercial microgreen tower used for production of sorghum sprouts and sorghum microgreen in larger quantities. Tower contains four levels with four trays each, 6 lights on each level, and each tray has 2 waterers.

Figure 5-2 Sorghum seeds being evaluated on the brown cotton substrate

Figure 5-3 Sorghum seeds being evaluated on the white cotton substrate

Figure 5-4 Sorghum seeds being evaluated on the burlap substrate

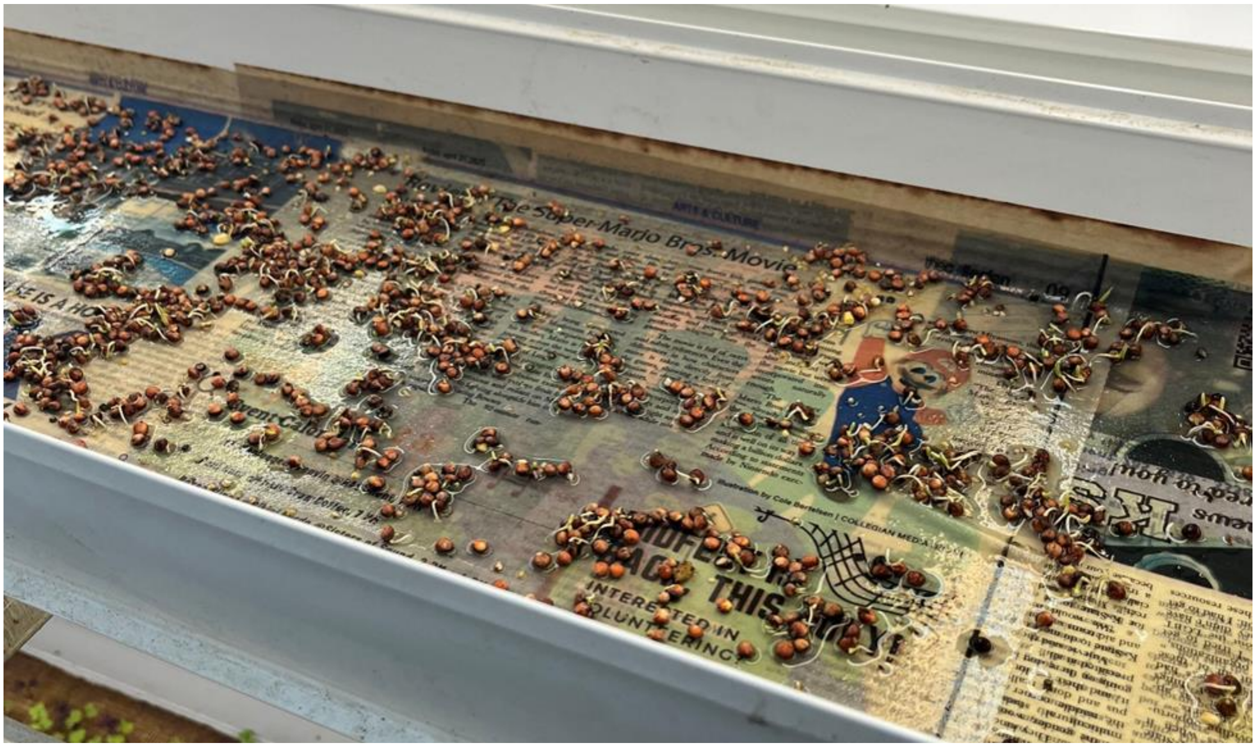

Figure 5-5 Sorghum seeds being evaluated on the newspaper substrate

Figure 5-6 Sorghum microgreens produced from each substrate compared in jars. Burlap is on the far left, newspaper on the middle left, white cotton on the middle right and brown cotton substrate on the far right.

Figure 5-7 Sorghum microgreens at 1.5 kg density

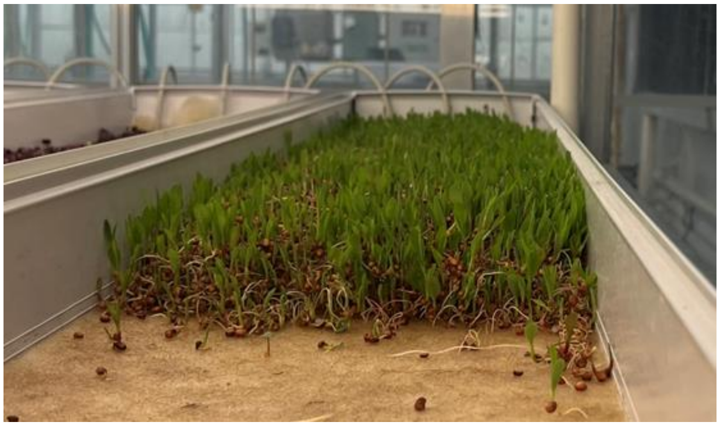

Figure 5-8 Sorghum microgreens at 0.68 kg density

Figure 5-9 Sorghum microgreen from 1.5 kg (left) and 0.68 kg (right) showing greenery growth versus root growth

Figure 5-10 Mold spots that were reported in the sorghum microgreens early in development

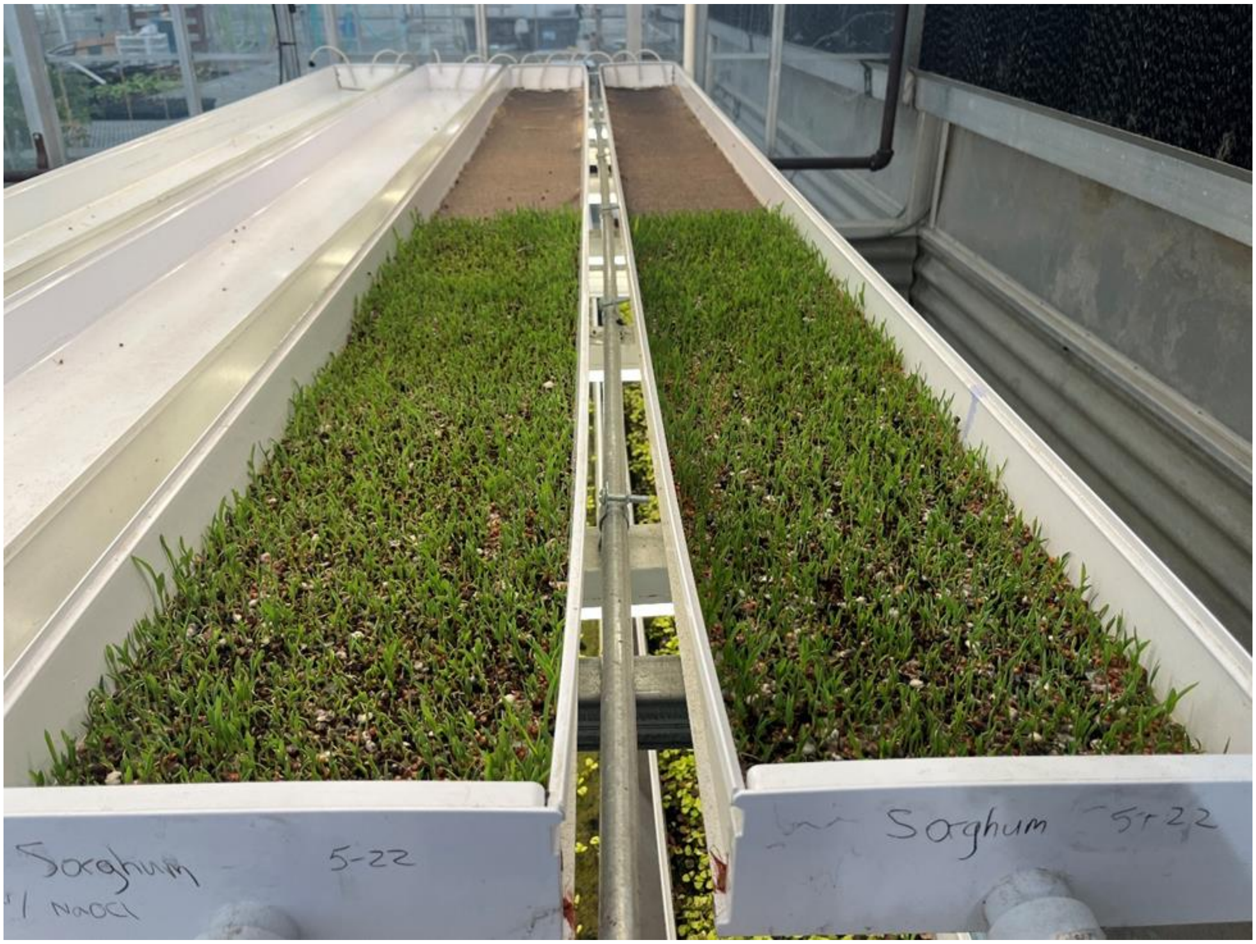

Figure 5-11 Sorghum with and without a pre-germination sodium hypochlorite solution rinse. Mold can be observed in both.

Figure 5-12 Final sorghum microgreen product after cleaning each piece of microgreen tower

Chapter 6 - Effect of sorghum sprouts and microgreens in raw diets on nutrient digestibility and fecal quality in healthy adult cats

Abstract

Raw diets have become more popular over the last few years. This usually entails a reduction in starchy ingredients like grains, tubers, and legumes. This is because these starches must be converted to glucose for proper utilization by cats through some process - typically cooking. Thereby negating the “raw” moniker. Germination may be an effective alternative that shifts a cereal grains nutritional bioavailability while retaining its unprocessed “raw” status. The objective of this study was to evaluate the apparent total tract digestibility (ATTD) of raw diets with germinated sorghum by healthy adult cats. The four dietary treatments evaluated were sorghum seeds that were cooked by boiling, water soaked, sprouted, and microgreen sprouts. The various sorghums were incorporated into diets at 30% and diets balanced to meet the NRC (2006) requirements for feline growth. Eleven healthy shorthair cats were allocated randomly to treatment and square in an incomplete replicated 4 × 4 Latin square design. Each experimental period consisted of a 5-day adaptation phase followed by a 5-day collection phase, during which total fecal collection and assessment of fecal score were performed. Data were analyzed using statistical software for a mixed model GLM (GLIMMIX procedure of SAS v. 9.4, SAS Institute, Cary, NC) with diet as fixed effect, and cat(square), square and period as random effects. During the trial, one cat was removed from period four due to reasons unrelated to the treatments, and two cats refused to eat the microgreen diet. Cats fed the microgreen and cooked diets exhibited lower ($P < 0.05$) wet fecal output compared to those fed the sprouts and raw diets. Fecal score was affected by diet ($P < 0.05$), with instances of loose stools and diarrhea more prevalent in cats fed the sprouts and raw diets. Stools were less frequent or not observed in the cats' fed microgreens or cooked treatments. Protein ATTD (avg. 90%) did not differ ($P > 0.05$) among dietary treatments. Dry matter, organic matter, and gross energy ATTD were 3-6% greater ($P < 0.05$) in cats fed the cooked diet compared to those fed the other treatments; but digestibility coefficients remained within acceptable ranges (> 80%). Fat ATTD was affected ($P < 0.05$) by diet, with cats fed the sprouts having the greatest value and those fed the cooked the lowest; however, the numerical differences (< 2%) were unlikely to have biological relevance. In conclusion, the microgreens and sprouted sorghum at 30% in raw meat cat diets did not improve the ATTD of energy and macronutrients compared to when sorghum was added in its raw form. Nevertheless, microgreen sorghum led to an improvement in fecal quality in cats compared to when sorghum was provided in its raw form.

Keywords: germination, ancient grain, carnivore, polyphenols

1. Introduction

In recent years, the greater interest in pet foods and their nutrition has led to pet owners selecting more "natural" and "ancestral" foods for their cats. This trend has many owners turning to raw diets. These raw diets are relatively unprocessed and typically consist of raw meat, bones, fruits, and vegetables (Ahmed et al., 2021). Grains are a good source of energy but often displaced in a raw diet since they require conversion by cooking to improve their utilization. Sorghum has some positive attributes as a choice of grains in a pet food because it is sustainable, non-genetically modified, gluten-free, and can be considered an ancient grain to support marketing claims (Xu et al., 2021, Chadalavada et al., 2021, *Sorghum: Ancient grain-timely solution*, n.d.). Furthermore, sorghum has high levels of polyphenols which may provide health benefits for the animals (Xu et al., 2021). However, in a pet diet raw grains need to be cooked and bound nutrients liberated. These include starch conversion to glucose, proteins into amino acids, and phytate into available phosphorus as examples (Ebert, 2022). These are often accomplished in conventional foods through cooking. In raw foods, a different approach is required since no portion of the diet can be subjected to heat in the course of preparation of food and still be deemed raw. (Association of American Feed Control Officials, 2017). Germinating seeds has been demonstrated to mobilize some nutrients and it would still qualify the food and ingredients as raw (Benincasa et al., 2019). While sprouts only contain a small white shoot emerging from the seed, microgreens are seeds that have a longer green shoot and contain nutritive benefits similar to sprouts (Bhaswant et al., 2023). Additionally, both sprouts and microgreens may impart immune benefits from compounds such as polyphenolic antioxidants (Wojdylo et al., 2020, Ebert, 2022). Germination has been reported to increase the total phenolic content of sorghum grain proving to be an-effective and promising avenue for antioxidant potentiation (Li et al., 2022). Sprouts and microgreens do differ in their benefits. Sprouts have been reported to have greater polyphenol content and greater levels of free amino acids (Wojdylo et al., 2020). Conversely, microgreens may have superior anti-diabetic, anti-obesity, and anti-cholinergic activities (Wojdylo et al., 2020). Despite the promising compositional work reported for sprouts and microgreens only a handful of studies have evaluated their effect on *in vivo* digestibility and health markers in animals (mostly livestock; Akinola et al., 2012, Afsharmanesh et al., 2012, Afsharmanesh et al., 2015). We hypothesized that adding sprouts or microgreens would make the raw diets more available to the enzymes for digestion compared to raw sorghum. Additionally, based on previous research of germination increasing polyphenols we believed it would also provide more polyphenols to the animal. To our knowledge, there are no reports evaluating their utilization in companion animals such as dogs or cats. Therefore, the objectives of this study were to determine the necessary steps to produce sorghum sprouts and microgreens in sufficient quantity to prepare diets for testing, and to determine the effect of sorghum sprouts and microgreens in raw diets on apparent total tract digestibility (ATTD) in healthy adult cats.

2. Materials and Methods

2.1 Seed Production

Sorghum was sourced from a regional grower that focuses on varieties suitable for human food products (NuLife Market, Scott City, KS). An accession of Burgundy sorghum was split into four different treatments: cooked, raw/soaked, sprouts, and microgreens. Microgreen and sprout treatments were grown using a commercial microgreen tower. All trays and materials were sanitized and washed prior to growth. A cotton substrate served as the base for each tray and 1.81 kg of sorghum seeds were weighed and spread uniformly on each tray. Waterspouts were set on automatic timers to dispense every two hours on 15 min intervals. Seeds were checked daily. Microgreens required 7 days and sprouts 2 days to grow to targeted endpoints (these lengths had been determined in preliminary testing). The timing of seed starts was coordinated accordingly such that the sprouts and greens would be ready the same day of raw diet production. Using gloved hands, seeds were carefully plucked from the cotton substrate to extract the whole seed (including tap root). A one-liter jar of sprouts/microgreens of composite sample from all trays were collected for analysis. Sprouts and microgreens were tested for Aflatoxin and Fumonisin and were determined to be below the safe limit for feeding (National Grain and Feed Association, 2011). The remaining seeds were collected and transported to a meat processing laboratory for experimental diet production. Concurrently, raw seeds were soaked overnight for 14 hours and then drained and transferred to the meat processing laboratory. The cooked sorghum was cooked 3:1 water to sorghum for approximately 45 min until the sorghum was soft and fluffy (Subramanian et al, 1981).

2.2 Raw Diet Production

Four nutritionally complete and balanced diets were formulated to exceed the recommendations for adult cat maintenance set by the Association of American Feed Control Officials (AAFCO, 2023). All diets were formulated to contain 30% of sorghum derived from the processes described above and 68% whole ground chicken, chicken liver and hearts sourced from a local grocery store (Hy-Vee, Manhattan, KS). The remaining 2% of the diet was comprised of a common basal mix sourced from a local mill (Wilbur Ellis, Clay Center, KS; Table 6-1). The meat processing lab complies with United States Department of Agriculture regulations for safe raw meat production. Using a meat grinder (Hobart, Model 4732, Troy, OH) all ingredients were ground to 1.6 mm and separated into separate containers. Once all ingredients were ground, they were weighed on a scale (Mettler Toledo, Model IND236, Columbus, OH) and placed into a mixer (Hollymatic Model 900E, Countryside, IL) and blended for 3 minutes in one direction, the rotation was reversed, and the contents were mixed for an additional 3 minutes until homogenous. The mixture was then weighed into 100–200-gram patties by hand and flash frozen in a -60°C freezer. The mixer was cleaned thoroughly in between each treatment.

2.3 Food Safety

Patties were individually vacuum sealed using a rollstock packaging machine (BullDog Ultrasource LLC, Kansas City, MO) and transferred to (Universal Pure, Lincoln, NE) coolers for High Pressure Processing (HPP) as a pasteurization step. Patties were processed for 300 seconds at 87,000 psi (600 MPa) – a method demonstrated to be sufficient to kill pathogenic bacteria of concern (Lee et al, 2023). Samples were evaluated at a commercial laboratory (Midwest Laboratories, Omaha, NE) approximately 10 days after being pasteurized and tested for aerobic plate counts, E. Coli, yeast, mold, *Staphylococcus aureus*, *Salmonella*, and *Listeria monocytogenes* using the following methods (AOAC 2015.13, AOAC OMA 2018.13, FDAA/BAM Chapter 18, AOAC 2003.07, AOAC OMA #2013.02 AFNOR QUA 18/03/15, AOAC RI 020401). All diets were negative for *Salmonella* and *Listeria* and considered safe for feeding.

2.4 Feeding Trial

All animal protocols were approved by the Kansas State University Institution of Animal Care and Use Committee (Protocol No. 4880). Eleven healthy adult American shorthair cats (6 male and 5 females) were enrolled in an incomplete 4x4 replicated Latin square design comprised of three squares, four treatments and four 10-day periods. Each of the four periods comprised 5-d of adaptation followed by 5-d of total fecal and urine collection (AAFCO, 2023). The average body weight of the cats was 5.07 ± 0.96 kg. Cats were fed twice daily to maintain body weight, based on estimates of energy requirements calculated from National Research Council (2006) where metabolizable energy was determined as $100^{*}\ BW_{kg}^{0.67}$. Raw diets were thawed in a refrigerator (4° C) and weighed into paper bowls immediately prior to feeding. Cats were allowed access to food for 1 h and all orts were collected and weighed, and bowls were discarded. Fresh water was available *ad libitum*. Feces and urine were collected twice daily. All feces were weighed and scored on a 1-5 scale in 0.5 increments where 1 was liquid diarrhea and 5 was dry hard pellets. A fecal score of 3.5-4.0 was considered ideal. One fresh sample (collected within 15 minutes of defecation) was collected from each cat per period, tested for pH and stored in a 2 mL tube and placed into -80°C for future analysis.

2.5 Nutrient Analysis of the diets and fecals

Diet and fecal samples were freeze dried (Harvest Right Scientific Pro freeze dryer, Salt Lake City, UT). Partially dried diet and fecal samples were analyzed for moisture (AOAC 930.15), ash (AOAC 942.05), fat by acid hydrolysis (ISO 11085:2008), crude protein (AOAC 990.03) using a nitrogen analyzer (FP928, LECO Corporation, Saint Joseph, MI), gross energy (Parr 6200 Calorimeter, Parr Instrument Company, Moline, IL), and total dietary fiber (AOAC 991.43). Nitrogen-free extract was calculated by subtracting all nutrients from 100.

2.6 Extraction of phenolics from raw samples and total phenolic content

Phenolic compounds in raw ingredient samples were extracted according to Lu et al. (2014) with modifications. Briefly, two grams of flour were extracted from each grain with 20 mL of 2M NaOH in 20% ethanol mixture in a 50 mL centrifuge tube. The mixture was left to shake in the dark for 3 h. Then, the pH was adjusted using concentrated HCl, and the mixture was centrifuged at 10,000 g, at 20 °C, for 15 min. The supernatant from each tube was collected and transferred to a new tube. The remaining mixture was extracted with equal amounts of ethyl ester and left to shake in the dark for 30 min. The tubes were then centrifuged at the same conditions and the supernatant was collected as previously stated. This step was repeated three times. The combined supernatant was evaporated using a nitrogen evaporator and stored at -20 °C until analysis.

The total phenolic content (TPC) assay was performed according to Tian and Li (2018) with modifications. The organic phase from the extraction phase was reconstituted with 3 ml of HPLC grade methanol in a 5 mL centrifuge tube. Digested supernatant (0.1 mL) was thoroughly mixed methanol (7.9 mL) and Folin-Ciocalteu reagent (0.5 mL). After 10 min, the mixture was added to Na_2Co_3 solution (20%, w/v). After resting in the dark for 2 h, the absorbance of the final mixture at 765 nm was recorded using a spectrophotometric plate reader (Synergy H1, BioTek Instruments, Inc., Winooski, VT.). Samples were analyzed using gallic acid as an external standard and expressed as microgram of gallic acid equivalence (GAE) per gram of the original sample (mg GAE/g).

2.7 Statistical Analysis

The feeding trial was conducted as an incomplete 4 x 4 replicated Latin square design. Each cat was randomly assigned to a treatment using a spreadsheet (Kim and Stein, 2009). Data was analyzed using the GLIMMIX procedure in SAS (SAS v. 9.4). Treatment was used as fixed main effects and cat (square), square, and period were considered random effects. Tukey's post hoc test was used to separate least square means where P values < 0.05 were deemed significant.

3. Results

3.1 Nutritional composition and total phenolic content of the raw ingredients

The raw sorghum and sorghum sprouts had greater dry matter content (DM; 63 and 74%) compared to cooked sorghum and microgreens (30-33%). Organic matter was consistent among the seed sources at 98%. Protein was lowest in the cooked sorghum and was greatest in the microgreen samples with ingredients ranging from 9-12.5% (DM). Raw sorghum and sorghum sprouts contained 3.5-3.8% (DM) fat, while cooked sorghum and microgreens contained around 2.4-2.9% fat (DM). Cooked sorghum contained the least amount of total dietary fiber (9% DM), while microgreens contained the greatest (17% DM). Insoluble dietary fiber was consistent with total dietary fiber in all samples, however raw sorghum and sorghum sprouts both contained greater levels of soluble dietary fiber compared to cooked sorghum and microgreens (0.6-0.7% vs. 0.1-0.2% DM).

The total phenolic content of the ingredients was reported on a dry matter basis. The soaked raw sorghum was analyzed to have 1,431 µg/g sorghum of total phenolics (Table 6-1). When sorghum was sprouted, it increased to 1,616 µg/g. However, the microgreen treatment contained over 4,200 µg/g of total phenolics. Whereas the cooked treatment only contained 780 µg/g of total phenolics.

3.2 Nutritional composition of the experimental diets

The raw and sprouted sorghum diets had a greater dry matter (DM) compared to the cooked and microgreen diets (Table 6-2; $P<0.0001$). Conversely, the cooked and microgreen diets had greater ash content than the raw and sprout diets (9.5 % vs 7.0-7.5 % DM; $P=0.0007$). Crude protein was different among all diets and was greatest in the microgreen diet (40 % DM) and lowest in the raw diet (33 % DM; $P<0.0001$). Fat was greater in the microgreen and cooked diet (22% DM, respectively) compared to the raw and sprout diets (19 % DM). Gross energy was greatest in the microgreen diet and lowest in the raw diet ($P=0.0007$). No differences were observed in total dietary fiber or insoluble dietary fiber among treatments ($P>0.05$). However, the cooked diet has greater soluble dietary fiber compared to the sprout diet (3.2 % vs 2.0 % DM). Nitrogen-free extract (NFE) was greater ($P<0.0001$) in the raw and sprouted diets (25%; DM) compared to the cooked and microgreen diets (14-15%; DM).

3.3 Food Intake and Stool Quality

Intake in grams per day (as is) did not differ among treatments (Table 6-3). Despite this, it should be mentioned that two cats were not removed from the study because of refusal to eat the microgreen treatment. Thus, the number of cats differed per treatment. Additionally, one cat was removed in period four due to dehydration, which is believed to be unrelated to the study. Thus, the number of cats for the cooked and sprouts treatments was 11, raw 10, and microgreens

9. The wet and dry fecal output were different among diets ($P<0.05$). Cats fed the raw and sprouts diets had greater fecal output on an as is basis (wet) compared to cats fed the microgreen and cooked diets ($P<0.0001$). When the feces were dried, the cats fed the raw diet still had the greatest output, while cooked had the lowest ($P=0.0004$). The number of defecations per day did not differ among treatments. Fresh fecal pH's (within 15 minutes of defecation) were greater (less acidic) in the cats fed the microgreen and cooked diets than those fed the raw and sprout diets ($P=0.0002$). Fecal scores did differ among treatments ($P<0.001$). The cooked and microgreen treatments mainly had fecal scores around 4 with microgreens resulting in a few in 4.5. However, the sprouts and raw containing diets had a lot more variance with over half of the cats having less than ideal fecal scores (58-60%). The raw sorghum containing diet resulted in 12% of cats having liquid, diarrhea-like stools, whereas the sprout containing diet contained mostly fecal scores slightly below ideal (score of 3 vs. below 3).

3.4 Apparent Total Collection Digestibility (ATTD)

Dry matter and organic matter ATTD were greater ($P<0.05$) in cats fed the cooked diet compared to the other diets. There were no differences in protein ATTD among diets (88-90 %, DM). Fat ATTD was greatest ($P<0.0001$) in cats fed the sprout diet (97 %, DM) and lowest in cats fed the cooked diet (95 %, DM). Nitrogen-free extract was greater ($P<0.0001$) in the cooked and microgreen diets (90-93%) compared to the sprouts and raw diet (75%). Gross energy ATTD was greatest ($P<0.0001$) in the cats fed the cooked diet (90 %, DM) compared to all other diets (86 %, DM).

3.5 Apparent Total Tract Digestibility (ATTD: Titanium Dioxide)

The apparent total tract digestibility using an external marker to estimate digestibility (titanium dioxide; 0.15% inclusion as is basis) is reported in Table 6-4. Dry matter and organic matter ATTD were greater (P<0.0001) in cats fed the cooked and microgreen diets compared to the cats fed the raw diet which was greater (P<0.0001) than the sprout diet. Protein ATTD was greater (P<0.0001) in cats fed the microgreen and cooked diets (89-90 %, DM) compared to the raw and sprout diets (86-87 %, DM). Cats fed the cooked diet had lower fat ATTD compared to the other three diets (P=0.0039). Nitrogen-free extract was greater (P<0.0001) in the cooked and microgreen diets (92-93%) compared to the sprouts and raw diet (58-63%). Gross energy ATTD was greatest in the cooked and microgreen diets, lowest in the sprout diet, and intermediate in the raw diet (P<0.0001).

4. Discussion

The objective of this study was to evaluate the ATTD of sprouts and microgreen sorghum in adult cats. This study is the first of its kind and therefore lacks published studies with companion animals for which to compare the results. Currently, most raw diets are meat-based and with the inclusion of some vegetables or fruits with starchy ingredients like cereals, tubers or legumes generally lacking. Mostly because these cereals require processing to liberate their calories through cooking (gelatinization) and contain some anti-nutritional properties (e.g., trypsin inhibitors) that are inactivated (denatured) by cooking. The process of cooking negates the ability to consider these diets raw. Thus, a method to convert these elements in starchy cereals would potentially open the range of ingredients available for use in raw diets and improve options to deliver health related molecules to pets. Some of those benefits come from the addition of total dietary fiber in a raw diet (Prasadi P. V. and Joye, 2020). Butowski et al. (2019) reported raw diets with the addition of plant-based fiber (2%; cellulose and inulin) resulted in a microbiome population more closely resembles a cat who eats kibble vs. one who eats raw, which may decrease the risk of pathogen exposure (Wernimont et al., 2020).

Germination allows grains to change nutritionally without compromising the diet’s raw status. In previous literature reports in which seed germination was evaluated the nutrients (starch, protein, phosphate) became more available to the digestive enzymes in the animal and were thereby more digestible (Bera et al., 2023, Gupta et al., 2015, Feizollahi et al., 2021, Durairajan et al., 2023, Chapter 4). Thus, the intention of this study was to determine incremental impacts from germination by selecting a zero (soaked) time point and two different levels of germination (sprout and microgreen) for their impact on ATTD in raw diets fed to cats.

The intention during formulation was that the diets would contain similar dry matter (moisture) contents, however, the outcome differed slightly from our plans. This was due to sprouts and raw sorghum not taking up a comparable amount of water to achieve the 60-75% moisture measured in the cooked sorghum and microgreens. This led to the differences observed in the dry matter in the ingredients, and diets. These changes were evaluated (described in Chapter 4) and it was determined that starch, phytic acid, fat, and soluble fiber were affected, but protein was not different based on processing type. Diets were formulated to be isonutritional based on the nutritional profiles from the preliminary study (Chapter 4). However, with the use of the microgreen tower the growth of the seeds greatly improved, and the protein content differed amongst our ingredients and carried through to the experimental diets. This differs from Ayeni (2021); wherein, they found that spinach and roselle microgreens at 20 days had decreased protein content compared to microgreens that were 10 days old. To the contrary, our findings agree with the results of Butkute et al. (2018) who reported microgreens had a greater protein content than raw seeds. Furthermore, Sibian et al. (2017) and Thakur (2021) also reported sprouts with elevated protein contents compared to their raw seeds. Additionally the dry matter among diets differed which led to differences in macronutrients among diets since each diet was formulated by overall weight rather than dry weight of the sorghum.

During the feeding trial, the food intake in grams per day was similar, however, two cats refused the microgreen treatment during the trial. Therefore, we acknowledge that palatability with this treatment was an issue and must be considered in the future. Cats are known to be finicky, and the inconsistent intake is often associated with their early kitten development with the Queen (Wexler-Mitchell, 2007). Thus, whether the intake was due to some inherently off-flavor or texture, or due to naivete surrounding this ingredient should be further evaluated. Due to the nature of previous research and findings in Chapter 4, it was expected that microgreens would have a greater ATTD compared to the raw treatment. However, our study reported similar ATTD between raw, sprout, and microgreens. This may have occurred since our raw seeds were not fully raw as a function of the soaking beforehand. This soaking was intended to equilibrate moisture content among seed treatments but may have inserted some unexpected bias. Previous studies have reported that soaking can decrease the phytic acid in seeds and reduce mineral and protein binding making them more available for digesting (Salim-Ur-Rehman et al., 2014). Sharma, R. and Sharma, S. (2022) found that soaking foxtail millet flour reduced phytic acid and improved *in vitro* starch and protein digestibility compared to the raw millet. In a similar study conducted by Sharma et al. (2023) white and red sorghum flour was soaked and was found to have lower levels of tannins, similar levels of phytic acid, and greater in vitro starch and protein digestibility compared to the raw sorghums. Despite the reduction in phytic acid and possible increases in protein and starch digestibility, it was still expected that the raw treatment would perform poorer than the microgreen and sprout treatments.

The cats fed the cooked diet had greater ATTD in dry matter, organic matter, and gross energy compared to those fed the other three diets. This differs from Kerr et al. (2012) who did not observe differences between a raw beef-based diet and a cooked beef-based diet in dry matter, organic matter, or gross energy when fed to domestic cats. The major difference is the lack of carbohydrates in their diet (95% beef; Central Nebraska Packing Inc., North Platte, NE) compared to this study (30% sorghum). However, the inclusion of cooked sorghum disqualifies this for classification as raw causing a dilemma for pet owners who want a truly raw diet. Cats fed sprouts had better fat ATTD compared to cats fed the other diets. This was different than Akinola et al. (2013) where pigs fed sorghum sprouts that resulted in lower fat digestibility compared to their corn-based basal diet. Major differences between the studies include inclusion rate of sprouts (23% vs 30%) and fat inclusion differences between diets (45-57% vs. 19-22%). Despite cats fed microgreens exhibiting comparable ATTD to those fed the raw sorghum-based diet, there is a notable contrast in fecal scores and fecal pH levels. Both cats fed sprouts and raw had lower fecal pH's than the microgreen and cooked diets. The raw sorghum treatment had a large proportion of fecal scores that were loose or diarrhea-like (31.67 %) compared to microgreens and cooked which were mainly firm and solid (cooked: 8.52 % loose stools, microgreens; 0%). Cats fed sprouts had 16.4% of the samples that were loose (< 3 fecal score) but did not qualify as diarrhea (< 2 fecal score) unlike cats that were fed the raw sorghum treatment. Additionally, cats fed the sprouts and raw each had greater fecal output (both dry and wet) than those fed the cooked and microgreens treatments which is undesirable for pet owners. This could mean that the animal is having trouble digesting the nutrients (Zoran, 2008), however, the results of our total collection data disagree with this. Ayeni (2021) reported that animals fed older microgreens (day 20 versus day 10) had greater carbohydrate digestibility compared to those fed younger microgreens. Therefore, microgreens may contain more digestible carbohydrates compared to sprouts. Our results corroborate this claim as the raw and sprouted based diets only had 75% NFE ATTD, whereas the microgreen and cooked based diets had 90-93% NFE ATTD. However, it should be noted that the raw and sprouted diets had 10% higher NFE on a dry matter basis compared to the microgreen and cooked diets. Additionally, Alvarenga et al. (2021) and Peixoto et al. (2017) reported that a greater resistant starch content led to a lower fecal pH. Raw sorghum had the greatest levels of resistant starch followed by sorghum sprouts in the preliminary study (Chapter 4) and may have been the cause of the lower fecal pH and fecal scores in cats fed those diets. Furthermore, Ezeogu et al. (2005) reported a difference in *in vitro* starch digestibility between raw and cooked sorghum which may have contributed to the fecal pH and scores in this study. The fecal pH of cats fed cooked and microgreen containing diets were similar to those from a study with corn fermented protein in an extruded diet (Kilburn-Kappeler et al. 2022) which reported that cats were healthy in a similar research setting. Overall, cats fed the raw and sprouted diets were numerically acceptable for ATTD (>80%), however, their stools were loose, and it was observed that the cats were struggling to fully utilize the nutrients in the diets. This aspect is particularly unattractive to consumers, as they prefer not to encounter an increase in feces to clean up and (or) want to manage liquid or loose stools. Cats fed microgreens and cooked treatments had firmer stools and more normal fecal pH levels compared to cats fed the raw and sprouted based diets.

In terms of health markers, polyphenols have been known to support gut health by enhancing the growth of beneficial bacteria, while reducing the available nutrient to the pathogenic bacteria (Wang et al., 2022). Sorghum contains high levels of polyphenols compared to other grains. Total phenolic content is an assay that can quantify all polyphenols in a sample.

Zhang et al. (2015), Kim et al. (2018), and Guardianelli et al. (2022) each reported an increase in total phenolic content after germinating buckwheat, wheat, and quinoa (red and white). Moreover, Liu et al. (2022) reported an increase in total phenolic content in 16 of 17 different seed types with alfalfa the only seed not conforming. This was confirmed in our study as the microgreen samples contained more than 2.5 times the amount of total phenolic content compared to sprouts, which was higher than our soaked raw treatment. However, cooking the sorghum diminished many of the phenolic compounds and reduced it to less than half the amount that sprouts contain. This is also consistent with a previous report by Mohapatra et al. (2019) who found that boiling sorghum decreased the total phenolic content compared to the raw sorghum grain. This boiling could be a function of leaching into the water phase that is decanted (Minatel et al., 2017). It was expected that the diets in this study would have 234-724 µg/g (30% of each ingredient on dry matter basis). The values were different than predicted. There is some question whether the polyphenols were affected by HPP. Previous studies have reported a steady or increasing concentration of total phenolics when HPP was applied at similar time and pressure settings compared to untreated samples (Lou et al., 2022, Fernandez et al., 2019, Chen et al., 2015). Thus, it is expected that total phenolic contents of the final diets were unchanged due to HPP.

Polyphenols are poorly absorbed with 5-10% estimated to disappear in the small intestine (Aravind et al., 2021). The remaining polyphenolics that appear in the colon may act as a prebiotic and provide benefits to the gut microbiome (Alves-Santos et al., 2020). Jewell et al. (2022) reported that an inclusion of 4% fiber bound to polyphenols in healthy adult cats had greater beneficial metabolites, lower levels of fecal branched chain fatty acids and ammonia and indicated a slight shift in the microbiome from a protein metabolism to a carbohydrate metabolism. Therefore, the high polyphenolic content in the microgreen diet could have influenced the stool consistency. However, due to the differences in macronutrients in our diets we are unable to conclude that polyphenols are the sole reason for the increase in fecal scores. Unfortunately, in the current study, blood metabolites, short chain fatty acids, or gut microbiome were not evaluated since the focus was on the larger question surrounding evaluating the digestibility of sprouts and microgreens in a raw diet and its ATTD in cats. Future work should be directed toward these other attributes. With these preliminary results qualifying the merit of sprouted and microgreen sorghum in a cat diets, future studies should evaluate parameters such as blood metabolites, fecal short and branched chain fatty acids, and the gut microbiome to determine the effects of sprouts and microgreen polyphenols on healthy adult cats and markers of health.

5. Conclusion

Raw diets with grains included reduce the amount of meat-based products needed, making them more cost-effective and sustainable. Microgreens contained over two and half times as much total phenolic content compared to all other treatments, while cooking decreased the amount of phenolics. The cooked sorghum-based diet was more digestible compared to the microgreen, sprouts, and raw sorghum diets. Despite the differences in diet ATTD, all digestibility coefficients were acceptable except for NFE ATTD in raw and sprouted diets which may be due to resistant starch. Cats fed the cooked and microgreen-based diets resulted in firmer stools and less fecal output compared to the raw and sprouted treatments. Further investigation is warranted to determine the impact of microgreens on palatability, blood metabolites, fecal short chain fatty acids, and the colonic microbiome to evaluate the overall potential health benefit for cats fed sorghum sprouts and microgreens.

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Table 6-1 Seed composition of sorghum used in experimental diets (dry matter basis)

Item	Raw/Soaked	Cooked	Sprout	Microgreens
Test Sorghum Composition (dry matter basis)				
Dry Matter, %	62.8	33.0	74.2	30.0
Organic Matter, %	98.0	98.6	97.9	97.6
Crude Protein, %	10.6	9.2	11.2	12.9
Fat, %	3.5	2.4	3.8	2.9
Total Dietary Fiber, %	10.3	9.3	11.7	16.8
Insoluble Dietary Fiber, %	9.6	9.1	11.1	16.7
Soluble Dietary Fiber, %	0.7	0.2	0.6	0.1
Ash, %	2.0	1.4	2.1	2.4
GE, kcal/g	4.4	4.4	4.3	4.4
Total Phenolic Content (µg/g ingredient)	1430.5	779.8	1616.4	4214.2

Table 6-2 Experimental diet ingredient composition and nutritional composition (dry matter basis)

Item	Raw/Soaked	Cooked	Sprout	Microgreens	SEM	P-value
Ingredient, % (as-fed basis)						
Sorghum	30.0	30.0	30.0	30.0		
Whole Ground Chicken	59.85	59.85	59.85	59.85		
Chicken Liver	4.0	4.0	4.0	4.0		
Chicken Hearts	4.0	4.0	4.0	4.0		
Dicalcium Phosphate	0.85	0.85	0.85	0.85		

Potassium Chloride	0.4	0.4	0.4	0.4		
Salt	0.25	0.25	0.25	0.25		
Choline Chloride	0.2	0.2	0.2	0.2		
Titanium Dioxide	0.15	0.15	0.15	0.15		
Trace Mineral Premix	0.05	0.05	0.05	0.05		
Vitamin Premix	0.05	0.05	0.05	0.05		
Taurine	0.05	0.05	0.05	0.05		
Magnesium Oxide	0.05	0.05	0.05	0.05		
Nutritional composition						
Dry Matter, %	38.9^{a}	31.0^{b}	36.7^{a}	31.3^{b}	0.87	<0.0001
Organic Matter, %	93.2^{a}	90.6^{b}	92.3^{a}	90.5^{b}	0.37	0.0005
Crude Protein, %	32.9^{d}	38.8^{b}	34.5^{c}	40.9^{a}	0.47	<0.0001
Acid Hydrolyzed Fat, %	19.0^{b}	22.1^{a}	19.1^{b}	21.7^{a}	0.54	<0.0001
Total Dietary Fiber, %	15.9	13.9	12.9	13.6	1.86	0.4419
Insoluble Dietary Fiber, %	13.5	10.7	10.9	11.1	1.83	0.4058
Soluble Dietary Fiber, %	2.4^{ab}	3.2^{a}	2.0^{b}	2.5^{ab}	0.30	0.0180
Nitrogen- Free Extract, %	25.3^{a}	15.9^{b}	25.8^{a}	14.3^{b}	1.95	<0.0001
Ash, %	6.8^{b}	9.4^{a}	7.7^{b}	9.5^{a}	0.54	0.0007
GE, kcal/g	5.27^{c}	5.44^{ab}	5.33^{bc}	5.55^{a}	0.051	0.0007
ME	3656	3790	3734	3777		

Calculated ME = [(3.5 x protein%) + (3.5% x NFE%) + (8.5% x fat %)} x 10

Table 6-3 Food intake, fecal parameters, and total collection digestibility of cats fed sorghum-based diets with sorghum processed different ways

Parameter	Raw (n=10)	Cooked (n=11)	Sprouts (n=11)	Microgreens (n=9)	MSE	P-Value
Intake (g/day, as is)	191	204	188	184	1.2	0.0662
Wet output (g/day, as is)	51.0^{a}	28.5^{b}	48.9^{a}	35.4^{b}	1.22	<0.0001
Dry fecal Output (g/day)	15.7^{a}	10.4^{c}	14.2^{ab}	12.0^{bc}	1.19	0.0003
Defecations per day	1.2	0.9	1.0	1.1	1.08	0.0787
Fresh fecal pH	5.02^{bc}	5.48^{a}	4.89^{c}	5.42^{ab}	1.049	0.0002
Total Collection Digestibility, %						
Dry Matter	78.79^{b}	83.32^{a}	79.70^{b}	79.20^{b}	1.145	0.0030
Organic Matter	82.34^{b}	88.38^{a}	83.00^{b}	84.20^{b}	1.117	<0.0001
Crude Protein	88.60	90.06	89.81	90.25	1.154	0.0728
Fat	96.56^{ab}	95.09^{c}	97.42^{a}	96.06^{bc}	1.277	<0.0001
Nitrogen-free extract	99.39^{b}	99.81^{a}	99.38^{b}	99.79^{a}	1.223	<0.0001
Gross Energy	85.10^{b}	89.65^{a}	86.01^{b}	87.03^{b}	1.126	<0.0001

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

MSE- mean squared error, N- number of cats per treatment

Table 6-4 Apparent total tract digestibility (titanium dioxide) of raw diets with sorghum processed different ways fed to adult cats

Digestibility, %	Raw (n=10)	Cooked (n=11)	Sprouts (n=11)	Microgreens (n=9)	MSE	P - Value
Dry Matter	76.47^{b}	82.42^{a}	72.60^{c}	79.72^{a}	1.126	<0.0001
Organic Matter	80.33^{c}	87.72^{a}	77.00^{d}	84.59^{b}	1.115	<0.0001
Crude Protein	87.35^{b}	89.49^{a}	86.21^{b}	90.52^{a}	1.023	<0.0001
Fat	96.08^{a}	94.83^{b}	96.46^{a}	96.21^{a}	1.010	0.0039
Nitrogen-free extract	99.11^{b}	99.82^{a}	98.94^{b}	99.81^{a}	1.319	<0.0001
Gross Energy	83.40^{b}	89.07^{a}	81.07^{c}	87.36^{a}	1.124	<0.0001

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

MSE- mean squared error, N- number of cats per treatment

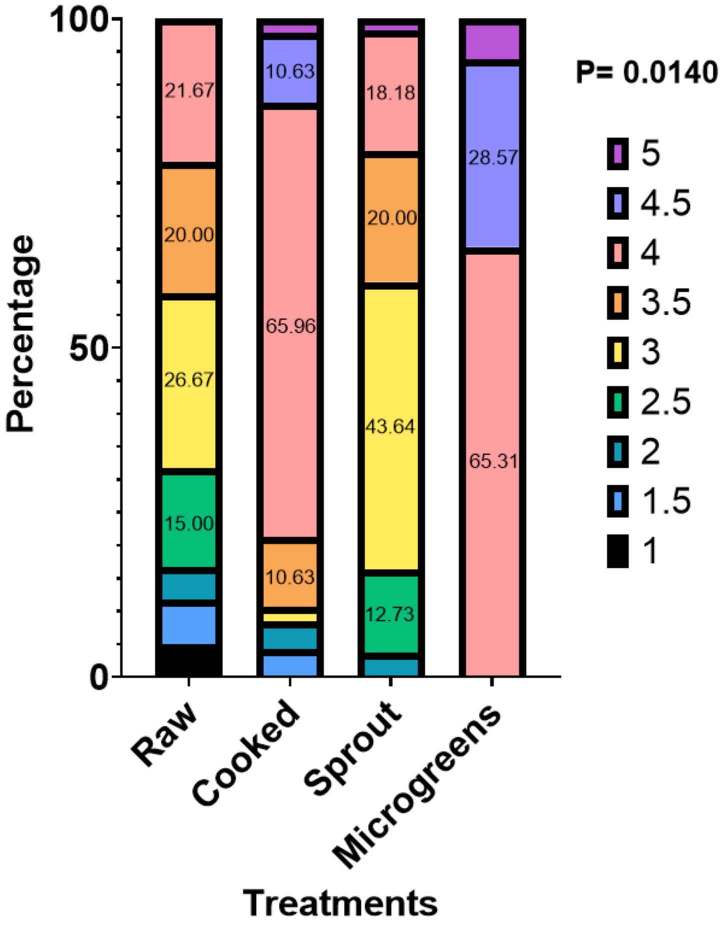

Figure 6-1 Fecal scores by frequency of cats fed experimental diets containing sorghum either raw, cooked, sprouted, or microgreens where 1= liquid diarrhea 1.5= liquid stool with slight consistency 2= soft, unformed stool 2.5= very moist, some retaining shape 33= moist, retains shape 3.5= firm, leaves a slight mark when picked up 4= firm, formed shape, does not leave mark when picked up 4.5 hard, dry stool, cracks when pressure is applied and 5= dry, hard, pellet like, crumbles when pressure is applied

Chapter 7 - Overall Summary and Future Research

Sorghum has many unique features that can be utilized in the pet food industry. However, its growth is hindered by outdated livestock literature and negative connotations. The aim of this thesis was to evaluate various sorghum accessions and preparations as an ingredient in extruded and raw pet foods for utilization by dogs and cats. The first study determined the processability of different sorghum pericarp varieties (white sorghum A, white sorghum B, burgundy sorghum, and sumac sorghum) compared to four small grains (rice, barley, oats, and millet). All four sorghum varieties produced well-expanded kibbles providing evidence for a good substitution for other small grains despite small differences in textural characteristics. Digestibility and oxygen radical antioxidant capacity (ORAC) of the sorghum containing diets were evaluated with 12 adult beagles in a 4 × 4 Latin square design experiment. Dietary treatments comprised 50% of white sorghum A or B, burgundy sorghum, or rice (as the control). No differences were observed in crude protein and fat apparent total tract digestibility ($P>0.05$). Furthermore, ORAC values were expected to differ as the total phenolic content on all sorghum treatments were more than twice the total phenolic content of rice, however, similar values were observed among all treatments ($P=0.4928$). This may be due to the overnight fast before blood was collected. A post-meal blood collection may be necessary to observe differences in dietary treatments. Dogs fed the rice diet had higher plasma cholesterol levels compared to dogs fed the three sorghum treatments ($P=0.0007$); otherwise, diet had little influence on humoral constituents. This suggests that sorghum may be a good substitute for rice in canine diets.

While kibble diets are the most common, fresh and raw diets have had the highest growth in the market in recent years. Additionally, consumers are demanding “natural” and less processed – even raw diets for their pets. Raw diets consist of raw meat, bones, fruits, and vegetables. However, they cannot undergo any heating to be considered “raw.” Because starches typically require cooking, they are not commonly included in raw diets. Germination may solve this problem as it mobilizes the nutrients within the plant. To evaluate this, five common cereal grains (corn, millet, sorghum, soybean, and wheat) were selected for evaluation. Seeds were germinated to initial sprout eruption and also allowed to grow into microgreens and then compared to the raw seeds. In general, a decrease in ash, fat (except for soybean), resistant starch (except for soybean), phytic acid (exception for corn and soybean) and increases in in vitro protein digestibility (exception for wheat) were observed in the germinated seeds compared to their raw seeds. From this, sorghum was selected to evaluate further for seed growth, diet processing, and animal utilization. Sprouts and microgreens were grown in a commercial microgreen tower (Throckmorton Hall, Kansas State University). Growth optimization was undertaken to determine the best substrate and seed density. Once a protocol was developed sprouts and microgreens were grown for food production. Raw diets containing 30% seeds were produced in the laboratory and then diets were pasteurized at high pressure (HPP) and verified for microbial safety at a commercial laboratory. Eleven shorthair cats were enrolled in a replicated incomplete 4 × 4 Latin square designed feeding study that consisted of 5 d adaptation followed by 5 d collection in each of the 4 periods. Treatments included raw sorghum, cooked sorghum, sprouts, and microgreens. Total phenolic content was measured in the raw ingredients and found that the microgreens had three times the total phenolic content compared to the raw sorghum, whereas the cooked treatment decreased almost 50% in total phenolics compared to the raw. Cats fed the sprouted and microgreen-based diets had similar dry matter, organic matter, and gross energy apparent total tract digestibility to the raw sorghum, and all were lower digestibility compared to the cats fed the cooked sorghum diet ($P < 0.05$). Among treatments, no differences were observed in protein digestibility (P> 0.05). However, fecal scores were the major difference in this study as cats fed the raw and sprouted diets had more diarrhea and loose stools compared to cats fed the microgreen and cooked diets. Furthermore, the microgreen diet had no loose stools or diarrhea which may be due to the high level of total phenolics measured. Polyphenols have been reported to positively impact gut health and therefore allow the cat to digest and absorb nutrients efficiently to produce ideal fecal scores. However, due to the differences in macronutrients we cannot conclude that polyphenols are the reason for the improved fecal scores. Future studies should utilize learnings from our work to improve true comparisons between experimental diets and the controls in order to evaluate the effects of polyphenols on fecal scores and the gut microbiome on cats (and dogs) fed sorghum microgreens or high polyphenol-containing diets. Additionally, future research could include evaluating the effects of a diet including sorghum microgreens on hematology, fecal short chain fatty acids, gut microbiome, and antioxidant capacity in dogs or cats. In extruded diets, further research should evaluate the effect of extrusion on the bioavailability of polyphenols in dogs and cats, new methodology to evaluate ORAC values in dogs and cats fed sorghum-based diets (post-feeding blood collections versus fasted blood), as well as evaluating sorghum bran, and potentially a stressed animal model fed a sorghum-based diet. Overall, sorghum is a good substitute for other common grains as it can process well in an extruded diet, provides high levels of polyphenols, and provides potential for new products in raw diets.