Benefits of Whole Grain Sorghum on Cat Health

Sorghum’s Impact on Gastrointestinal, Skin and Coat Health

As proposed by:

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Summary

Obesity, a common problem in the U.S. cat population, leads to impaired regulation of blood glucose and insulin as well as an increase in inflammation and oxidative damage. Inflammation and oxidative damage may lead to reduced skin and coat quality as well as intestinal disturbances that negatively impact stool quality. Research has demonstrated that novel dietary ingredients can reduce inflammation-driven diseases. As a result, some pet food companies are pursuing the identification of novel ingredients to suppress diseases in companion animals. Based on our observations that sorghum-based diets alter colon microbiota, fecal characteristics (including moisture and short chain fatty acid content) and inflammatory state, in addition to reducing the estimated glycemic index of foods, we aim to investigate the potential of sorghum as a novel ingredient with the potential to improve feline health. The overall goal of the proposed work is to determine the impact of sorghum grain on skin and coat condition, and intestinal and metabolic health of cats consuming diets containing sorghum grain. To address this goal, we will evaluate the following three objectives in cats consuming a controlled diet made with rice or experimental diets made using white, black or sumac sorghum grain:

1) Determine the skin and coat quality before and after consuming diets containing the selected sorghum grains.
2) Determine plasma glucose and insulin prior to and after consuming diets containing the selected sorghum grains.
3) Characterize fecal microbial populations, fecal short chain fatty acids and metabolomic profiles prior to and after consuming diets containing the selected sorghum grains.

Background and Relevance

The importance of meeting feline nutritional requirements and justification for proposing to study the effect of sorghum on intestinal health and for skin and coat quality in cats are based on multiple observations in the literature and those from our laboratory. Skin and coat quality and appearance are important indicators of pet health, and both of these visual attributes are sensitive to the nutritional status of the pet (Vester and Fahey, 2006). An increase in inflammation has been noted to increase dermal issues in cats. Changes in metabolism that lead to altered skin characteristics can lead to changes in skin pH and increased water loss, making it possible to use transepidermal water loss and pH as indicators of skin integrity (Vester and Fahey, 2006). Contributors to colon disease include microbial dysbiosis (Turner et al., 2013), altered microbial metabolism (Hamer et al., 2012), aberrant signaling (Candela et al., 2011), as well as perturbation of colonocyte gene expression (Cho et al., 2012; 2014). We have demonstrated that sorghum brans, especially those containing elevated levels of polyphenolic molecules, are capable of reducing colon cancer (Turner et al., 2009) and the injury induced by chronic inflammation (Ritchie et al., submitted). These effects may be occurring because of changes in the microbiota and their short chain fatty acid metabolites (Turner et al., 2010; Ritchie et al., submitted; Ritchie et al., in preparation).

Obesity is a serious disease in cats (German, 2006). It contributes to development of diabetes, which is similar in nature to human type 2 diabetes where insulin resistance develops (Butterwick and Hawthorne, 1998; Zoran, 2010; Zoran and Rand, 2013). Although difficult to remedy in cats, it is imperative to reduce blood glucose and the need for elevated insulin release. One way to achieve this would be to increase resistant starch in diets containing grains, which would limit starch digestion and glucose absorption in the small intestine. We have demonstrated an increase in resistant starch content of porridges prepared with polyphenol-rich sorghum grains and their fractions/extracts (Austin et al., 2012; Lemlioglu-Austin et al. 2012), suggesting inclusion of polyphenol-rich sorghum grains in feline diets may reduce the elevations in glucose and insulin observed after a meal.

There are an estimated 74 million cats in the U.S. (de Godoy et al., 2013). Health challenges in feline populations include obesity, diabetes, intestinal disorders and poor skin and coat quality, all of which are associated with an elevated inflammatory state. Research has demonstrated the potential for diets to be involved in these maladies, and thus pet health. A growing number of individuals wish to
provide unique, novel and “natural” food ingredients to their cats in an attempt to improve pet health and longevity (Sloan, 2014). The drive behind this desire is the recognition that some problems with intestinal disorders and dermal pathologies may be lessened with novel ingredients that are not routinely found in pet foods, which contain beneficial bioactive compounds.

Several companies have evolved to address the natural pet food ingredient desires of customers. One of the oldest natural pet food companies, Nutro (one of the international Mars Petcare Companies), has produced premium natural pet foods for nearly a century. Nutro has a history of conducting the research necessary to develop nutritional claims for food ingredients, which provide nutritional benefits for pets. Among their current research program is a focus on the benefits of whole grains, with two priorities being gastrointestinal health and skin and coat quality. Based on our observations that sorghum-based diets improve colon health and microbial populations, Nutro is interested in investigating the potential of sorghum as a novel ingredient with the potential to improve feline health.

Currently, 79 percent of pet owners consider the quality of their pets food to be as important as their own, which explains why 40 percent of the $26 billion pet food market in the U.S. is made up of the premium sector and 10 percent is made up of the super-premium sector (Sloan, 2014). The natural product sector is a large component of the premium pet food market, making it a significant commercialization opportunity. Nutro is an industry leader in natural pet foods with primary research goals, which include improving skin and coat quality and intestinal and metabolic health in cats. Collaborating with Nutro provides an opportunity to introduce them to sorghum. Based on our experience with sorghum, we anticipate deriving health benefits for cats, which could motivate inclusion of sorghum into their natural product lines. Expanding the global market for sorghum grain into the natural pet food market would significantly increase demand for sorghum in the U.S. and other countries where the demand for premium pet foods is rapidly growing.

Justification and Relevance to Target Audience

The work proposed addresses the need for improved feline diets capable of meeting the nutritional needs for healthy skin and desirable coats as well as preserving intestinal and metabolic health. Considerable effort is put into ingredient discovery that meets not only the nutritional requirements of cats, but that also provide additional benefits derived from the biologically active compounds with antioxidant activity and the capacity to alter glucose metabolism and intestinal microbiota.

Goals and Objectives

The long-term goal of this research program is to enhance use of sorghum grain in high quality cat foods, with the aim of improving pet health. To achieve this goal, the current project has the following overall goal and specific aims.

Overall Goal: To determine the impact of sorghum grain on hair and skin condition and intestinal and metabolic health of cats consuming diets containing sorghum grain.

Specific Objectives:
1. Determine the skin and coat quality before and after consuming diets containing the selected sorghum grains.
2. Determine plasma glucose and insulin prior to and after consuming diets containing the selected sorghum grains.
3. Characterize fecal microbial populations, fecal short chain fatty acids, and the metabolomic profiles prior to and after consuming diets containing the selected sorghum grains.

Methods/Activities

The animal component of the project will be conducted at a Mars Petcare Facility in England using their standardized animal care procedures (Hewson-Hughes et al. 2011), while the biological sample analyses will be conducted at Texas A&M University.
Animals, Experimental Design, Feeding: The trial will utilize 14 adult domestic shorthaired cats fed four diets (control white rice, white sorghum, black sorghum, sumac sorghum) in a crossover design, equally distributing cats of the same hair color between the different groups. All test diets will be extruded dry food, formulated to provide equal amounts of grains to meet nutritional and energy requirements for adult maintenance. Each cat will have a full veterinary examination before the start of the trial. All cats will receive NUTRO® NATURAL CHOICE® Wholesome Essentials Cat Food during the pre-feeding and washout periods. The cats will be offered food on an energy per group bodyweight basis (60 kcal/kg BW) and their body weights monitored. Each treatment period will last six weeks.

Sample Collections/Analyses and Qualitative Measure Methods

Daily group intake: The energy requirement of each cat will be calculated at the beginning of each feeding stage and the amount offered adjusted accordingly. Group food intakes will be recorded daily for each of the control and test panels. Individual weekly body weights will be measured to monitor and correct any over or under-feeding.

Sensory Evaluation of Coat Condition: The Quantitative Descriptive Analysis (QDA) panel is composed of five technicians who have been selected and trained in house to provide accurate and precise assessments of skin and coat quality. Assessments are performed in a uniformly lit room to avoid variability in scores due to different environmental conditions. Coat gloss is measured before any manual examination of the animal is carried out. The amount of light reflected from the coat is used to assess gloss. Coat softness is a measure of the feel of the coat when the assessors run fingers through the full thickness of the coat. An optimum coat feeling is obtained with the absence of either a greasy or a dry feeling (as often the two are indistinguishable on the basis of feel alone) and is measured at the same time as the softness. Scale (dander) on the animals’ coat is an undesirable quality and is measured by visually assessing the amount of scale present in six different sections of the coat. Each of the four variables will be assessed in each cat twice at the end of the pre-feed (week 8 and week 9) and twice at the end of the test phase (week 17 and week 18). The panel members use a scale from one to five and are able to easily differentiate 0.25-0.50 units.

Skin pH: Five consecutive skin pH readings will be taken from the dorsal patch shortly after shaving. This will repeated at the end of each test phase. Skin pH measurements will also be taken from the hairless inner surface of both the left and right ear.

Transepidermal Water Loss: Skin hydration will be evaluated by measuring the conductivity of the skin using a dermal phase meter, which yields a direct measure of the hydration of the stratum corneum. Continuous measurements taken over a 30 second period provide a direct measure of trans-epidermal water loss. Measurements will be taken in duplicate at the end of the pre-feed stage and at the end of the test phase from the inner surface of both left and right ears.

Stool Quality: Stool quality will be evaluated using a scale of 1-5. A score of one represents no solid form and more than 75 percent liquid; two is used to describe feces that is soft and approximately 50 percent liquid; three represents feces that retains some cylindrical shape and is more than 75 percent formed; a score of four represents feces that retains more than 75 percent of the cylindrical shape and if more than 50 percent was firm; a score of five will be used if it is cylindrical in shape and more than 80 percent is firm (Hall et al., 2013). Multiple assessments will be made during each treatment period and an average value generated for each animal that will be used for analysis of the effect of diet.

Plasma Glucose and Insulin: Blood samples will be collected using a cannula inserted into the cephalic vein prior to and at the end of each diet period. Plasma samples will be analyzed for insulin (EDTA tube) using the porcine insulin RIA kit (PI-12K; Millipore) in stored samples (-80°C), while glucose (Flu-Ox tube) will be assayed immediately with an automated analyzer (AU400; Beckman Coulter).

Intestinal Health: Feces being collected prior to starting each treatment diet and at the end of
the treatment period. These samples will be used to determine how diet impacts the microbiota present, their metabolic profile, and SCFA concentrations. Samples will be processed using procedures described below.

**Microbial DNA isolation:** Aliquots of the fecal samples will be weighed in cryovials, flash frozen in liquid N2 and stored at -80°C. The frozen feces will be thawed on ice and homogenized in ice-cold PBS using a FastPrep procedure (MP Biomedicals) for two minutes. DNA will be extracted from the resulting homogenate (500 mL) using the FastPrep-24 homogenizer and the DNA Spin Kit for Feces (MP Biomedicals). DNA concentration will be determined using spectrophotometry and samples will be stored at -20°C for sequencing analyses (Ritchie et al., in preparation; Ritchie et al., submitted).

**DNA library preparation and bar coding for deep sequencing:** One nanogram of intact genomic DNA will be processed using the Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, CA). Target DNA will be simultaneously fragmented and tagged using the Nextera Enzyme Mix containing transposome that fragments the input DNA and adds bridge PCR (bPCR)-compatible adaptors (5'-AATGATACGGGCAGTCTACACAGCTCTCTCGCAGATCTACACGCCTCCCTCGCCATCAG-3' and 5'-CAAGCAGAAGACGGCATACGAGATCGGTCTGCCTTGCCAGCCCGCTCAG -3') required for binding and clustering on the flowcell. Next, fragmented and tagged DNA will be amplified using a limited-cycle PCR program. In this step index 1(i7) and index 2(i5) (combination will be unique to each sample) will be added between the downstream bPCR adaptor and the core sequencing library adaptor (Fadrosh et al., 2014), as well primer sequences required for cluster formation (Primer 1: 5'-AATGATACGGGCAGTCTACACAGCTCTCTCGCAGATCTACACGCCTCCCTCGCCATCAG-3’, Primer 2: 5'-CAAGCAGAAGACGGCATACGAGATCGGTCTGCCTTGCCAGCCCGCTCAG-3’). The thermal profile for the amplification will have an initial extension step at 72°C for three minutes and initial denaturing step at 95°C for 30 seconds followed by 12 cycles of denaturing of 95°C for 10 seconds, annealing at 55°C for 30 seconds, a 30 seconds extension at 72°C, and final extension for five minutes at 72°C. The DNA library will then be purified using Agencourt® AMPure® XP Reagent (Beckman Coulter, Indianapolis, IN). Each sample will be quantified and normalized prior to pooling. The DNA library pool will be loaded on the MiSeq reagent cartridge (Illumina, San Diego, CA) and on the MiSeq instrument (Illumina, San Diego, CA). Automated cluster generation and paired-end sequencing with dual reads will be performed.

**SCFA analysis:** The SCFA analyses will be conducted using our standard protocols (Zoran et al., 1997; Crim et al., 2008; Ritchie et al., submitted). Fresh samples are flash frozen and stored at -80°C. Frozen samples are ground in liquid nitrogen, mixed with an internal standard (2-ethylbutyric acid), and extracted in 70 percent ethanol. Extracts are centrifuged and aliquots of supernatant are removed and mixed with another internal standard (heptanoic acid) prior to injection onto a HP-FFAP column in a Varian 3900 GC. Concentrations in the samples are determined by comparison to a commercially available mix of standards.

**Metabolomics:** The metabolic profile of the feces will be determined to evaluate if consumption of the sorghum containing diets influence overall metabolic patterns of the microbiota. Metabolomic profiling will provide an opportunity to understand how the diet treatments affect the relative level of more than 2,000 metabolites. In order to perform this analysis, 100 milligrams of feces will be placed in polypropylene tubes before being frozen in liquid nitrogen and stored at -80°C. Samples will be analyzed in collaboration with Metabolon, an industry-leader in metabolomics (Gall et al., 2010; Sreekumar et al., 2009; Wetmore et al., 2010). The analyses will be performed using three independent platforms: UHPLC-MS/MS (+ESI), UHPLC-MS/MS (-ESI), and GC-MS (+EI). The mass spectra generated will be compared to a database of known standards. Metabolon will provide the raw data including biochemical name, super pathway, sub pathway, KEGG ID, HMDB ID and amount detected for each compound. This information will be used to perform statistical and bioinformatics analyses that will provide insight into the complex biochemical processes being affected by consumption of the sorghum containing diets.
Statistical Analyses: All results will be presented as the change in parameters from the end of the pre-feed to the end of the test phase. Statistical differences between the changes observed in each diet will be assessed by one-way ANOVA. Analyses of the microbial populations and the metabolomics datasets will use a false discovery rate protection procedure.

Timetable: First, we will optimize diet preparation conditions to assure a quality food product prior to initiating the feeding studies. It will require the next 50 weeks to complete the four diet periods. Sample processing and analyses will proceed upon collection with the goal in mind of completing all analyses by two years.

Evaluation
As part of the management plan, we will have quarterly conference calls between the pet research facility coordinator in England, Preston Buff at Nutro, and the Texas A&M University, team of investigators. We will continually evaluate progress using the proposed timeline.

Education/Outreach
Graduate and undergraduate students in Dr. Turner’s laboratory will conduct the proposed work. Information gained through this work will be included in class lectures, in seminars presented at other institutions/agencies, and at pet nutrition scientific meetings in the U.S. and internationally. Time will be made to accommodate the need to give lectures to targeted audiences made up of individuals who are considered thought influencers that carry messages to the industry and to consumers. In addition, the data will be published as abstracts and as manuscripts.

References


