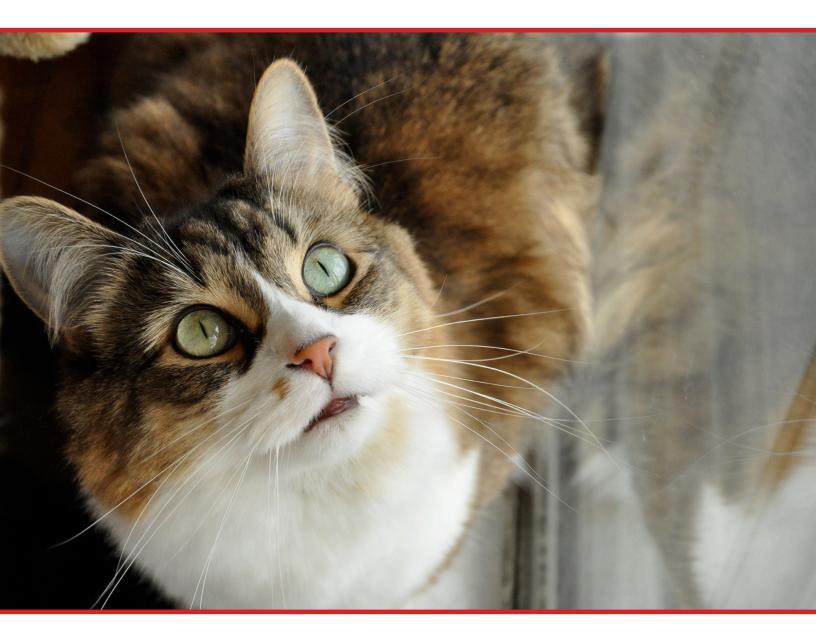
Use of Grain Sorghum as the Primary Ingredient in Premium Extruded Foods Designed for Cats



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Summary

Grain sorghum is an underutilized crop, especially for value-added applications such as foods for humans and pets. One important reason for underutilization of sorghum in human food and pet food alike are prevalent 'misconceptions' and lack of scientific data on the nutritional quality and also acceptability (or palatability) of sorghum-based products. This study aims to generate such scientific data with regard to use of sorghum in pet food products. Pet food production is a multibillion dollar industry in the U.S. with continuous growth for the past 10 years. Pet food sales are estimated to reach \$21.26 billion during 2013. There is potential for use of grain sorghum in pet food products as a less expensive alternative to other cereal ingredients such as corn and rice. Sorghum can also provide nutritional benefits related to slower digestibility of starch or lower glycemic response, which can aid in premium products targeted towards obese, diabetic and geriatric (or old) pets. The drawbacks of sorghum related to poor digestibility of protein and bitter taste due to tannins can be addressed by using a high-energy and dry processing method such as extrusion. This project was designed for validating the above hypotheses in relation to use of sorghum in dry expanded cat food. The overall objective of the proposed project is to develop nutritionally balanced and highly palatable premium pet food products for cats with grain sorghum as the primary cereal ingredient and carbohydrate source. These premium pet foods will be targeted towards obese cats where the resistant and low glycemic nature of sorghum starch would be especially attractive, while utilizing a balanced formulation and the special 'dry processing' characteristic of extrusion to provide adequate and bio-available amino acid profile and palatability.

Background and Relevance

There are approximately 384 million companion animals in the U.S. which is greater than the human population. Just under half (178.9 million) of the total number of pets falls under the classification of cats and dogs (APPA, 2013). The pet food industry is a multi-billion dollar industry with continuous growth for the past 10 years. Common pets foods contain between 30-60 percent carbohydrates, primarily from grains (De-Oliveira et al., 2008; Murray et al., 1999). Only a limited number of pet food brands, which utilize sorghum, e.g. Eukanuba and Iams, as the primary source of starch. This could be because sorghum inclusion in pet food has not been justified as efficacious due to dearth of scientific evidence, and also considering the issues of starch and protein digestibility in sorghum. On the other hand, corn and rice is widely used for cat food production. Sorghum and corn have similar nutrient profile with slight differences. The effectiveness of different cereals as source of starch in pet food foods has not been studied in relation to processing, especially for cat foods. Protein digestibility of sorghum is a big issue primarily because 70 percent of sorghum protein is made up of kafirins that are tightly bound in protein bodies.

Cats, which are the focus of this study, have a shorter digestive tract with about one third of the size of other animals who are natural herbivores. Thus protein digestibility of sorghum-based foods is a concern that needs to be addressed. Majority of dry pet foods, greater than 95 percent, are made using extrusion processing (Spears and Fahey, 2004; Tran et al. 2008; Gibson and Alavi, 2013). Extrusion cooking improves safety, digestibility and bioavailability of nutrients in grain based foods, including pet foods (Kienzle, 1993; Camire, 1998). As compared to other cooking methods that involve relatively large amounts of water, extrusion has been shown to disrupt sorghum protein bodies due to low moisture based dry cooking characteristics, high shear and high temperature treatment, thereby increasing protein digestibility (Fapojuwo et al. 1987; Hamaker et al., 1994). On the other hand, amylose-lipid complexes are also formed during extrusion, which might inhibit digestion of starch and provide associated benefits (Biliaderis, 1991; Lin et al., 1997; Gibson and Alavi, 2013; Thombre et al., 2004). For pets such as cats, besides meeting basic nutritional needs, health challenges, such as body weight and metabolism related problems, are important issues. Changes in lifestyle, neutering and new food habits have led to an increase in the incidence of metabolic diseases, obesity, colitis and others in cats, disturbances that potentially can be ameliorated by fiber or slowly digestible carbohydrates in the diet (Nelson et al., 2000).

There is currently an upswing in pet overweight and obesity throughout the world with reported incidence rates of up to 58 percent for cats (and 53 percent for dogs) in the U.S. (Phillips-Donaldson

2014; McGreevy et al. 2005). Various other diseases are associated with obesity, such as high blood pressure, joint problems and respiratory issues. Obesity can not only lead to glucose intolerance and insulin resistance but also to dyslipidemia and possible onset of diabetes in cats and other mammals (Kelly and Wills, 1996; Wolever and Bolognesi, 1996; Nguyen et al., 1994; Jordan et al, 2008; Villaverde and Fascetti, 2014). However, in a limited number of studies involving cats, six comparatively small serum glucose and insulin variations have been reported after consumption of different starch and sugar types (Kienzle, 1994a, Bouchard and Sunvold, 2000; Appleton et al., 2004). Only one publication has evaluated use of sorghum for cats in relation to glucose and insulin response (de-Oliveira, et al., 2008). Despite speculations, scientific studies so far have not focused on evaluating the use of less or slowly digestible starch in control of glucose intolerance and metabolic disorders in cats. This is an important focus of the proposed study.

Resistant starch (RS), the sum of starch and its degradation products not absorbed in the small intestine but fermented in the colon, has been considered an interesting prebiotic (Muir and O'Dea, 1992; Thompson, 2000; Lobo and Silva, 2003; Polesi, 2011). Reduction of glucose and insulin concentration in plasma had been verified after RS consumption in some animal species (Zhou et al., 2008; Regmi et al., 2011) and human beings (Raben et al. 1994). It is hypothesized that the nature of sorghum starch granules favors generation of under gelatinized and resistant starch during processing, thus increasing the potential of sorghum as source of an important prebiotic with significant health benefits for pets.

Part of the RS effect on carbohydrate metabolism is attributed to short-chain fatty acids (SCFA) formation by microbial fermentation in the gut. SCFA allow production and release of the incretin hormones, which have further strong influence on carbohydrate metabolism (Deng et al., 2013). Among the incretin hormones, GLP-1 (glucagon-like peptide-1) and tyrosine-tyrosine peptide (PYY) are produced by specific cells of the colon mucosa, delay gastric emptying, and thus promote satiety (Holst, 1997). The PYY peptide also acts directly on the hypothalamus and stimulates the release of other hormones that induce satiety (Batterham et al., 2002). Thus, increased gut fermentation activity induced by resistant starch consumption from sorghum-based diets and elated production of SCFA may induce satiety in cats. Increased secretion of GLP-1 and PYY may also favor glucose control (Wen et al., 1995; De Graaf et al., 2004).

While sorghum might have nutritional benefits that need to be explored, it could present challenges with regard to flavors such as bitterness that are potentially addressed by volatilization during extrusion. Descriptive sensory analysis by trained human panels is an important new method to analyze pet food products (Di Donfrancesco et al., 2012; Koppel, 2014). Combination of results from physicochemical testing, descriptive sensory analysis, palatability testing and consumer studies (including both pet owner and the pet) could provide a complete understanding of acceptability of sorghum-based food products by cats and that is another important focus of this study.

Pet food production is a multi-billion dollar industry in the U.S. with continuous growth for the past 10 years. Pet food sales are estimated to reach \$21.26 billion during 2013. There is potential for use of grain sorghum in pet food products as a less expensive and more sustainable alternative to other cereal ingredients such as corn and rice. Sorghum can be used in premium products targeted towards obese cats, which will provide nutritional benefits related to slower digestibility of starch or lower glycemic response and higher SCFA formation and absorption in the gut. The drawbacks of sorghum related to poor digestibility of protein and bitter taste due to tannins can be addressed by using a high-energy and dry processing method such as extrusion in combination with alatants designed specifically to mask undesired flavors and enhance acceptability. This project is conceptualized for validating the above hypotheses in relation to use of sorghum in dry expanded cat food.

Justification and Relevance to Target Audience

Sorghum remains one of the cheapest cereals that is abundantly grown in the U.S. Its application in pet food manufacturing has the potential to increase returns for sorghum growers. However, there is a need for further scientific facts on the efficacy of sorghum as a primary carbohydrate

source in foods designed for cats. The objective of the proposed project is to produce high quality dry expanded cat food products using sorghum as a grain base and compare these diets with rice and corn-based cat foods. The sorghum-based cat food will be nutritionally adequate and will be targeted for cats that require low-energy density foods. The sorghum-based cat food will have a lower glucose and insulin response in the cat. The diets produced will be highly palatable and acceptable to a cat panel. It is anticipated that this study will lead to a high quality, low energy and low glycemic product for cats using sorghum as the primary cereal ingredient. Overall, the expectation is to increase the awareness of sorghum as a novel and high value ingredient for pet food applications, and eventually increase returns to various stakeholders especially sorghum farmers.

Goals and Objectives

The overall goal of this project is to develop nutritionally balanced and highly palatable premium dry extruded foods for cats using sorghum as the primary grain ingredient and carbohydrate source. These premium pet foods will be targeted towards obese or obesity prone cats. The specific objectives of this study include:

1) Development of balanced formulations for extruded dry cat food with low tannin (white) and high tannin (red) sorghum varieties as a primary grain ingredient, and a rice and corn-based formulations as the controls.

2) Milling of sorghum varieties to varying particle sizes and their utilization in production of dry expanded cat food kibbles using extrusion processing.

3) Physico-chemical characterization, resistant starch quantification and in-vitro digestibility studies for extruded cat food.

4) Descriptive sensory evaluation using a human panel, development of suitable palatants, and palatability testing in an industry-setting cattery.

5) Evaluation of in-vivo digestibility and fermentation in the colon (measurement of short chain fatty acids in feces) of sorghum-based diets in comparison with a standard rice and corn-based diets using laboratory cats

6) A consumer/ home trial with cats to investigate the effectiveness of selected premium products, long-term diet acceptance by cats and owners, food intake behavior, animal and owner attitudes to diet and dietary management, the perception of health, vitality, wellness, and food satiety of the animals.

7) In depth study for comparison of selected sorghum-based diets with corn-based control diet in laboratory cats with regards to postprandial glucose and insulin response, satiety, and the production of the incretin hormones that control satiety such as glucagon-like peptide-I (GLP1) and tyrosine-tyrosine peptide (PYY).

Methods/Activities

Premium dry, extruded, low energy foods will be designed for obese cats by utilizing the resistant nature of sorghum starch. The overall approach is described in detailed below.

Objective One - Formulation of cat food: Four separate diets will be formulated for typical dry expanded cat food. Different varieties of sorghum will be used as the primary carbohydrate source for two of the diets. As a control, the third and fourth diets will be based on corn and rice, respectively. The basal formulation used for the sorghum based diet is shown in table 1 and will be adjusted according to the actual composition of the various ingredients. The corn and rice-based control diets will be similarly formulated and will be iso-nutritional with respect to sorghum diets based on fat, protein and fiber.

Objective Two – Milling and extrusion: The grain sorghum will be ground into three different particle sizes using a hammer mill with screen sizes of 0.4, 1.0 and 1.6 mm, and mixed with other raw ingredients which will be milled separately (0.4 mm screen). Dry expanded cat food kibbles will be produced by extruding the diets on a pilot-scale single screw extruder (X-20, Wenger Manufacturing, Sabetha, KS) at an in-barrel moisture of 28 percent wet basis, followed by drying and cooling using a continuous gas-fired dryer (Series 4800, Wenger Manufacturing, Sabetha, KS). The extruder conditions will be adjusted to achieve three different thermal: mechanical energy ratios.

Thermal and mechanical energy input during extrusion will be varied based on the total amount of steam input (thermal energy) and extruder screw speed (mechanical energy). This will lead to a 2x3x3 experimental design for extrusion with two sorghum varieties, three sorghum particle sizes, and three processing conditions. In addition, the control rice-based and corn-based diet will also be extruded. Thus a total of twenty treatments will be extruded and evaluated for physic-chemical characteristics and in vitro digestibility.

Table one:	Basal	diet for	extruded	cat food
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Ingredient	Quantity (%)	
Low tannin sorghum	42.5	
Chicken byproduct meal	35.0	
Poultry fat	7.0	
Corn gluten meal (60 percent CP)	9.0	
Liquid palatant	3.0	
Beet pulp	2.0	
Salt	0.45	
Potassium chloride	0.35	
Premix min/vit*	0.30	
Choline chloride	0.25	
Mold inhibitor	0.10	
Antioxidant	0.04	

*Added oer kilogram of diet: vit A, 15,000 IU; vit D3 1,300 IU, thiamin 10 mg; riboflavin 14 mg; pantothenic acid 60 mg; niacin 90 mg, pyridoxine 9 mg; folic acid 0.50 mg; vitamin B12 0.2 mg; iron 130 mg; copper 13 mg; magnesium 13 mg; zinc 180 mg; iodine 2 mg; selenium 0.3 mg

Objective Three – Physico-chemical characterization and in vitro digestibility of extruded cat food: Extrudate physical characteristics such as expansion ratio, bulk density, piece density, durability and hardness will be determined using standard methods. Proximate composition (protein, fat, ash, total dietary fiber, carbohydrates, moisture content and total calorie) of the manufactured cat foods will be determined using standard methods according to AOAC (2010). A differential scanning calorimeter (DSC) will also be used to determine degree of starch gelatinization and amylose-lipid complexation (Budde, 1952; Gibson and Alavi, 2013). A second method based on glucoamylase enzyme treatment will also be used to confirm the extent of starch gelatinization (Gibson and Alavi, 2013). The concentration of the resistant starch will be determined according to the Association of Official Analytical Chemists (AOAC) method 2002.02. In vitro analyses for organic matter disappearance (OMd) in extruded diets will be carried out as described by Hervera et al. (2007 and 2008). OMd can be used as a predictor of the digestible energy content of extruded cat foods. In vitro digestibility studies will also be conducted for evaluation of resistant, rapidly and slowly digestible starch.

Primary and secondary screening of extruded cat food treatments: Based on results from objective three (physic-chemical characterization and in vitro digestibility) as described above, products will be screened and eight sorghum-based diets will be selected, along with the rice and corn-based controls, evaluated for descriptive sensory attributes and palatant development (objectives 4a and 4b) and in vivo performance (objective 5). Thus, a total of 10 treatments will be evaluated in objectives 4a, 4b and 5 using the methodology described below. Treatments for palatability study (objective 4c) will be fewer (6) and will be selected based on secondary screening of cat food products based on results from objective 4a and 4b (descriptive sensory and palatant development) and objective 5 (in vivo studies).

Objective Four – Sensory evaluation, palatant development and palatability studies.

Descriptive sensory and aroma profile evaluation: Five highly trained panelists will receive

further orientation on dried cat food before proceeding with sensory tests. Appearance, texture, flavor and aroma attributes will be studies as per standard procedure. The panelists will be asked to chew one kibble for flavor and texture evaluation on a 15-point scale. A second round of descriptive sensory tests will also be conducted on a limited number of treatments after coating of products with palatants for the palatability testing (Objective 4c). Gas chromatography will be used for studying the volatile aroma compounds extracted using the headspace-solid phase microextraction (HS-SPME) method (Koppel et al., 2013).

Palatant development: Descriptive sensory profile of products will be used to develop palatants suitable for enhancing acceptability of the sorghum-based extruded cat food diets and masking undesired flavor and aroma attributes. This part of the study will be conducted in the facilities of SPF (France).

Palatability studies: Extruded sorghum-based diets and the controls will be coated with fat and suitable palatants, and evaluated for first preference (product consumed first) and palatability (product consumed in larger amount) using the two-pan method (Griffin, 2003) with 38 individually housed cats of different breeds and body weights over two consecutive days. The cat food kibbles will be first coated with oil and palatants specially developed for the sorghum-based products in objective 4b. To determine cat food palatability, the consumption rate, using the two-pan test method, will be calculated by the formula: Relative consumption (%) = (Food Aconsumption x 100)/ (Food A1consumption+Food B consumption)

Objective 5 - In vivo digestibility, and fermentation profile: The procedure for in vivo studies will be as described in Fisher et al. (2012). Three blocks of 20 adult health cats, from 5-8 years old, and ideal body condition score will be used. The experiment will follow a randomized block design, with three blocks of 20 cats, two cats per diet in each block, and six cats per diet in total. During the experiment, cats will be kept in individual stainless steel metabolic cages (90 cm x 90 cm x 100 cm), equipped with a system to separate feces and urine for collection. The amount of food offered will be calculated according to the energy value of food and energy requirement of the animal. In each block period, diet adaptation will occur from days 1-10, total collection of feces and urine from days 11-17, and fresh feces collection (within maximum time of 15 min of elimination) for short-chain fatty acid (SCFA), lactic acid and ammonia determination and pH measurement from days 18 to 20. Diet and fecal samples will be analyzed for dry matter, crude protein, ash, starch, total dietary fiber and acid-hydrolysed fat using standard methods. Gross energy content of diets, feces and urine will be determined using a bomb calorimeter. Fresh fecal samples will be used to measure pH and determine fermentation products including short-chain fatty acids (gas chromatography), lactic acid (colorimetric method) and ammonia (distillation in a nitrogen system).

Final screening of extruded cat food treatments: Based on results from Objective 4c (palatability studies), products will be further screened and two sorghum-based diets will be selected and, along with one control corn (or rice) based diet, used in consumer and home trials (Objective 6) and in an in-depth study for postprandial hormonal responses and satiety (Objective 7). These three diets will be produced in a second round of extrusion experiments and formulations may be adjusted for nutritional, sensory and palatability attributes, depending on results from previous objectives. Thus, a total of three treatments will be evaluated in objectives six and seven.

Objective six – Home and consumer trial: A total of 40 homes and owners per treatment will be recruited in the Kansas City area for this two-month duration study. The study will be designed to investigate the effectiveness of selected premium products, long-term diet acceptance by cats and owners, food intake behavior, animal and owner attitudes to diet and dietary management, the perception of health, vitality, wellness and food satiety of the animals. The study will measure the quantities consumed by the pet animals and weight changes, and the results will be reported by the animal owners.

Objective seven – Postprandial insulin and hormonal responses and satiety effect of the diets: An in depth study will be conducted for comparison of two sorghum-based diets with the corn or rice based control diet in laboratory cats with regards to a) postprandial glucose, insulin and

the incretin hormones Glucagon-Like Peptide-I (GLP1) and tyrosine-tyrosine peptide (PYY) response of the cats as described by de-Oliveira et al. (2008) and b) satiety of cats after being fed the experimental diets. Inferences about the satiety of the animals will be made by the consumption of a challenge super premium meal compared with the experimental foods as per standard procedure.

Evaluation

The expectation is that this study will provide further understanding of the use of sorghum as an ingredient in pet food manufacturing, which will in turn represent a strong potential for new applications of sorghum in an otherwise new territory. The pet food industry is a multi-billion dollar market world-wide, and the constant need for alternative and affordable ingredients drives innovation in the industry. Sorghum is cheap and it is produced in abundant quantity in the U.S., especially in Kansas. This niche use for sorghum when proven scientifically to be efficacious will definitely open new markets both locally and internationally for sorghum growers.

Education/Outreach

Results from the study will be widely disseminated to stakeholders, including sorghum producers, ingredient suppliers, pet food manufacturers, process equipment manufacturers, pet owners, scientific community and the general public via presentations of scientific information in national and international meetings such as the Pet Food Forum and Institute of Food Technologists Annual Meeting; publications in peer-reviewed scientific journals such as Journal of Animal Feed Sciences and Technology; and popular articles in Pet Food Industry, Cereal Foods World and others.

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