SOYBEAN HULLS AS A DIETARY FIBER SOURCE IN CANINE AND FELINE DIETS

BY

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THESIS

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ABSTRACT

Soybean hulls (**SBH**) are a fiber-rich co-product of the soybean oil extraction process that corresponds to 8% of the soybean seed. Despite being readily available and priced competitively, SBH are underutilized in monogastric nutrition. Therefore, two studies were conducted to evaluate the use of SBH as a dietary fiber in canine and feline diets. Four diets were formulated with either SBH, beet pulp (**BP**), or cellulose (**CL**) as the main source of dietary fiber (15% total dietary fiber), with the last diet formulated with no supplemental fiber (**NF**). All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. The studies were replicated 4x4 Latin square designs. Each period consisted of 14 d, with 10 d of diet adaptation followed by 4 d of total fecal and urine collections. At the end of each period, a blood sample was collected and analyzed for serum chemistry. Food was offered twice daily and fed to maintain body weight. In the first study, eight adult female beagles (mean age = 4.6 ± 0.6 yr; mean BW = 12.8 ± 1.7 kg) were used. Food intake (g/d) on a dry matter basis (**DMB**) did not differ among treatments. Fecal score was lower ($P < 0.05$) in dogs fed CL (2.0) in contrast with other dietary treatments (mean = 2.3), using a 5-point scale. As-is and DM fecal output did not differ in dogs fed BP, CL, or SBH and values were approximately 50% greater ($P < 0.05$) than dogs fed NF. Apparent total tract (**ATT**) dry matter, organic matter, and gross energy digestibilities were greater ($P < 0.05$) in dogs fed NF when compared to dogs fed BP, CL, or SBH. Dogs fed CL had greater ($P < 0.05$) ATT fat digestibility (94%) compared with all other treatments (mean = 91%). Dogs fed CL and NF had greater ($P < 0.05$) ATT crude protein digestibility, 87% and 86%, respectively, while SBH and BP resulted in intermediate (83%) and lower (79%) digestibility coefficients. Fecal total short-chain fatty acid (**SCFA**) concentration was greatest ($P <$ 0.05) in dogs fed BP (582.5 μmole/g) and SBH (479.7 μmole/g) when compared to NF and CL (267.0 and 251.1 μmole/g, respectively). In the second study, eight adult male cats (mean age = 10.5 yr ± 0.1; mean BW = 6.1 kg ± 0.8 kg) were used. Food intake expressed on a DMB was lower ($P < 0.05$) in cats fed BP (55.2 g/d) when compared to SBH (70.8). Fecal score was higher ($P < 0.05$) in cats fed the NF diet (2.8) compared to the three fiber treatments (mean = 2.2) on a 5-point scale. As-is fecal output did not differ in cats fed BP or SBH and, when expressed on a DMB, fecal output did not differ between fiber treatments. Apparent total tract dry matter, organic matter, and gross energy digestibilities were greater ($P < 0.05$) in cats fed NF when compared to those fed BP, CL, or SBH. Cats fed CL had greater ($P < 0.05$) ATT crude protein digestibility (89%), while cats fed NF and SBH had intermediate digestibility (85 and 82%, respectively) and those fed BP had the lowest (77%). Cats fed CL had greater ATT fat digestibility (93%) than cats fed BP (87%) and SBH (89%). Total dietary fiber ATT digestibility was lowest in cats fed NF and CL (9 and 15%, respectively), followed by SBH (18%) and cats fed BP having the highest digestibility (33.7%). Total SCFA concentration was greatest in cats fed BP (699.7 μmole/g) when compared to the other three treatments, while phenol and indole concentrations did not differ among treatments. In both animal studies, there were no effects of dietary treatment on serum metabolites and all animals remained healthy throughout the studies. In conclusion, SBH resulted in similar ATT macronutrient digestibilities when compared to BP and CL; fiber sources widely used in commercial pet foods. Therefore, SBH could be a viable dietary fiber source in canine and feline diets.

To My Family

Thank you for your endless support, encouragement, and love.

I could not have accomplished this without you.

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TABLE OF CONTENTS

CHAPTER 1:

INTRODUCTION

Pets have become an integral part of the family, with approximately 74.1 million cats and 70 million dogs in American homes, according to the most recent pet census (AVMA, 2012). As a result, owners have developed a strong bond with their pets and are seeking ways to increase the health and longevity of their pets. This has caused a renewed interest in dietary fibers in companion animal diets to mirror the owner's own health practices. Dietary fiber is not nutritionally required by an adult cat or dog (NRC, 2006); however, it can provide a multitude of benefits. Depending on the fiber source, added fiber in companion animal diets can improve gut health, improve glucose homeostasis, improve fecal quality, dilute caloric density, and may increase short-chain fatty acid production (Banta et al., 1979; Fahey et al., 1992; Massimino et al., 1998; Swanson et al., 2002; den Besten et al., 2013).

Soybean hulls (SBH) are a fiber-rich co-product of the soybean oil extraction process and account for 8% of the soybean seed (Gnanasambandam and Proctor, 1999). Soybean production in the United States has steadily increased followed by a steady decrease in production cost (USDA, 2017). This has resulted in SBH being readily available. However, minimal research has been done evaluating the effects of SBH in canine diets when compared to other dietary fiber sources commonly used in pet foods (i.e. beet pulp and cellulose). Previous research has evaluated various sources of SBH fed to dogs with varying concentrations of total dietary fiber (TDF) and insoluble to soluble ratios (Cole et al., 1999; Burkhalter et al., 2001). However, TDF values did not exceed 9% in those diet formulations.

Due to the scant scientific research pertaining to the nutritional and functional relevance of SBH in canine and feline diets, the objectives of these studies were to determine the effects of

SBH on food intake, apparent total tract nutrient digestibilities, fermentative end-products, and fecal quality when fed in high TDF-containing canine and feline diets.

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CHAPTER 2:

LITERATURE REVIEW

PET POPULATION AND PET FOOD INDUSTRY IN THE U. S.

In the 2012 pet census, published by the American Veterinary Medical Association (AVMA), there were approximately 70 million pet dogs and 74.1 million pet cats in the U. S. (AVMA, 2012). By 2017-2018, the National Pet Owners Survey reported 89.7 million pet dogs and 94.2 million pet cats, which totals to 68% of U.S. households owning a pet (APPA, 2018). Not surprisingly, the U.S. pet industry expenditures have mirrored the growth of the pet population as well. Expenditures have grown from $48.35 billion in 2010 to an estimated $69.36 billion in 2017, with $29.69 billion being in pet food alone (APPA, 2018). Within pet food sales, large retailers (i.e. Target, Walmart) are responsible for over 70% of dog food sales while pet superstores have a 20% market share, followed by small pet supply stores (Case, 2014).

As a result of the high number of pets in households, owners have developed a strong emotional bond with their pets and seek to find ways to increase the health and longevity of their pets, as well as mirroring the owner's own health practices. This has led to a paradigm shift in the industry, moving from trying to simply achieve nutritionally adequate diets to innovations in food format (i.e. freeze-dried, raw, dehydrated), packaging, niche products, and health claims (Case, 2014). Following this shift, the popularity of grain-free products, products of U.S. origin, organic products, and minimally processed foods have received increasing interest.

There also has been a renewed interest in dietary fibers in the pet food industry (de Godoy et al., 2013). Dietary fiber is not nutritionally required by an adult cat and dog (NRC, 2006). However, they provide many health benefits. In pet foods, dietary fibers can be used to dilute caloric density, blunt postprandial glycemia and control glucose homeostasis, improve gut health and fecal quality, and increase the production of short-chain fatty acid (SCFA), which are used as an energy source by the colonocytes and host (Banta et al., 1979; Fahey et al., 1992; Massimino et al., 1998; Swanson et al., 2002a; den Besten et al., 2013) Dietary fibers also contribute to satiety, which may aid in weight loss and thus mitigate concurrent diseases associated with obesity (German, 2006). In a clinical survey conducted in 2016 by the Association for Pet Obesity Prevention (APOP), 53.9% of dogs and 58.9% of cats were classified as clinically overweight or obese (APOP, 2016). Obesity is a risk factor for a multitude of diseases, such as osteoarthritis, diabetes, cancer, and cardiovascular disease (Kealy et al., 2000; Van Gaal et al., 2006). There have also been studies in human nutrition indicating that added dietary fiber provides health benefits, including lowering risk of coronary heart disease, glycemic control, obesity, and high cholesterol (Brown et al., 1999; Wolk et al., 1999; Papathanasopoulos & Camilleri, 2010). While dogs and cats are resilient to the development of cardiovascular disease, dietary fibers can be used as an effective strategy to support pet health and wellbeing.

DEFINITION AND CLASSIFICATION OF DIETARY FIBERS

Dietary fiber is most commonly defined as the edible part of plants or analogous carbohydrates resistant to digestion and absorption in the small intestine, with complete or partial fermentation in the large intestine (Trowell et al., 1985). However, the definition of dietary fiber has remained fluid in that it continues to change and evolve as more research on the subject arises. The Food and Drug Administration (FDA) recently revised the approved definition of dietary fiber, as non-digestible soluble and insoluble carbohydrates (with three or more monomeric units) and lignin that are intrinsic and intact in plants, and isolated or synthetic nondigestible carbohydrates determined by the FDA to have beneficial physiological effects to human health (FDA, 2016). There are also several ways dietary fiber can be classified. Tungland and Meyer (2002) classified dietary fiber components based on their role in the plant, type of polysaccharide, gastrointestinal solubility, site of digestion, and physiological classification. But, the most common classification of dietary fiber is dividing them into two different categories: insoluble and soluble (Dai and Chau, 2016). Fiber contains insoluble components (i.e. cellulose, select hemicelluloses, and lignin) and soluble components (i.e. pectins and gums). Total dietary fiber (TDF) is composed of these two fractions. Soluble fibers have high water holding capacity, slows the rate of nutrient absorption, and delays gastric emptying (Serena et al., 2008). Insoluble fibers are included in diets to decrease intestinal transit time, bind organic compounds, increase fecal bulk, and promote laxation (Renteria-Flores et al., 2008). In human nutrition, the addition of soluble fiber to the diet has been used to reduce the glycemic response in both healthy subjects as well as patients with diabetes mellitus (Jenkins et al., 2008). The insoluble to soluble fiber ratio plays an important role in how nutrient digestibility and fermentative end-products will be affected when fed in monogastric diets. Additionally, depending on the fiber source, the extent and rate they are fermented in the large intestine can vary. This will result in different concentrations of fermentative end-products produced, including short-chain fatty acids (SCFA) and putrefactive compounds [i.e. phenols, indoles, branched-chain fatty acids (BCFA)] (Dai and Chau, 2016).

Dietary fiber has traditionally been associated with impaired nutrient utilization, reduced net energy, and decreased performance of poultry and swine (Janssen and Carré, 1985; Stewart et al., 2013; Lowell et al., 2015). However, in humans, fiber has been linked to having a multitude of benefits, including positive influences on diseases such as colon cancer, type II diabetes, and obesity (Anderson et al., 1994). Thus, due to owners wanting to mirror their own health practices when choosing a pet food, dietary fiber has become of interest in companion animal diets. Research has highlighted that added fiber can improve intestinal and host health. Health benefits of dietary fiber include improved fecal characteristics, nutrient utilization, laxation, and production of SCFA (de Godoy et al., 2013). *In vitro* studies have been conducted using fecal inoculum from dogs and cats to evaluate the degradation of different fiber sources and, in some cases, to model fiber fermentation kinetics over time (Swanson et al., 2002b; de Godoy et al., 2009; Panasevich et al., 2013; de Godoy et al., 2015; Myint et al., 2017). Sunvold et al. (1995a) evaluated the effects of different fiber sources on SCFA production using *in vitro* fermentation with dog and cat fecal samples used as inoculum. The four fiber sources evaluated were cellulose, beet pulp, citrus pulp, and citrus pectin. Measurements were taken at 6, 12, 24, and 48 h. At 48 h, cellulose had the lowest total SCFA production in both dogs and cats (0.08 and 0.02 mmol/g, respectively), followed by beet pulp (5.28 and 5.73 mmol/g), citrus pulp (5.52 and 6.68 mmol/g), and citrus pectin (6.16 and 7.48 mmol/g). While the largest differences were observed at 48 h, a similar pattern was also observed at 6, 12, and 24 h. Coincidentally, a linear increase in SCFA production was parallel to the amount of fermentable components each of the fiber sources contain. Results from *in vitro* fermentation using cat fecal inoculum indicated that the substrate fermentation profile and the amount of SCFA produced is dependent on the composition of the fiber (Sunvold et al., 1995b). Additionally, in an *in vitro* study conducted by Myint et al. (2017), the effects of cellulose and soybean husk (synonymous to soybean hulls) on fermentative end-product concentrations were evaluated using dog fecal samples as the inoculum. Incubation of soybean husk produced a greater ($P < 0.01$) amount of total SCFA when compared to cellulose. Additionally, lower ($P < 0.01$) concentrations of indoles were detected for soybean husk. The study's *in vitro* results indicated that soybean husk could beneficially affect fermentative end-products when added as a dietary fiber in canine diets.

COMMON FIBER SOURCES IN COMPANION ANIMAL DIETS

Dietary Fiber in Canine Diets

Beet pulp and cellulose are considered the most common fiber sources in companion animal diets (de Godoy et al., 2013). Beet pulp contains both soluble and insoluble fibers and is considered the gold standard in commercial pet foods. However, there can be inconsistencies within beet pulp sources (i.e., TDF content and soluble:insoluble fractions). Reported beet pulp TDF values have ranged from 60.1 to 76.8% (Fahey et al. 1990a,b). Cellulose is highly pure, which reduces variability. Reported cellulose TDF values have a much smaller range of 91.6-99.9% (Sunvold et al., 1995a,b,c; Swanson et al., 2002b; Fischer et al., 2012; Kerr et al., 2013). However it primarily consists of insoluble fibers of poor fermentability. Sunvold et al. (1995c) found that moderately fermentable fiber sources (12.5% beet pulp equating to 8.9% TDF) promote desirable stool characteristics without compromising nutrient digestibility or gastrointestinal health of the dog.

The optimal inclusion level of beet pulp in extruded canine diets was evaluated by Fahey et al. (1990). Fecal excretion responses and mean retention time were evaluated when comparing varying levels of beet pulp inclusion on a percent basis. Thirty female English Pointers were fed isonitrogenous diets containing 0, 2.5, 5.0, 7.5, 10.0, or 12.5% beet pulp (n=5). The beet pulp used consisted of 16% viscous polysaccharides, 31% hemicelluloses and non-viscous polysaccharides, and 25% cellulose. This combination of viscous and non-viscous components allows beet pulp to be a moderately fermentable fiber. The results of that study concluded that beet pulp levels up to 7.5% inclusion rate appeared as an acceptable dietary fiber source in canine diets. In this experiment, 7.5% beet pulp inclusion in the diet, which equated to 12.0% TDF. This optimal level of beet pulp (7.5%) has been used as a positive control in other experiments and compared against other fiber sources. For example, three different levels of oat fiber inclusion were compared to a no fiber control and beet pulp. It was found that oat fiber up to 7.5% inclusion performed similarly to a 7.5% beet pulp diet in terms of wet fecal weight, fecal dry matter, frequency of defecation, digesta mean retention time, and apparent total tract (ATT) nutrient digestibility (Fahey et al., 1992).

Additionally, there have been different fiber sources tested in canine diets and compared to beet pulp. Fahey et al. (1990) evaluated beet pulp, tomato pomace, peanut hulls, wheat bran, and alkaline hydrogen peroxide-treated wheat straw in diets for adult dogs. All fiber sources were included in the diet to achieve a TDF value of 12.5%. All four of the diets behaved similarly when compared to beet pulp for ATT nutrient digestibility and metabolizable energy content. However, fecal metabolites (i.e. SCFA, BCFA, phenols, and indoles) were not measured in this experiment, so it is difficult to conclude whether the fiber sources tested were comparable to beet pulp in terms of optimal fermentability and gastrointestinal health.

Muir et al. (1996) evaluated the effect of dietary fibers in terms of fermentation characteristics. Five ileal cannulated dogs were fed high-protein, high-fat diets that were supplemented with either 0% supplemental fiber, 7.5% beet pulp, a low-cellulose mixture (2.5% cellulose and 5.0% pectin), a high-cellulose mixture (5.0% cellulose and 2.5% pectin), or cellulose (7.5% cellulose) using a 5 x 5 Latin square design. Total dietary fiber values for the dietary treatments were 2.6, 8.6, 9.7, 9.7, and 8.7%, respectively. In terms of apparent ileal digestion, all treatments had similar digestibility values, with the exception of fat that was lower

($P < 0.10$) in the beet pulp treatment group compared to the three cellulose treatments (93.2% vs. avg. 95.2%). The low-cellulose mixture diet had lower apparent ileal fat digestibility in comparison to the high-cellulose mixture diet and the cellulose diet. Apparent total tract digestibility of dry matter (DM), organic matter (OM), and gross energy (GE) were lower in the fiber-containing diets compared to the control diet. Even though the increased cellulose incorporation had little effect on ileal nutrient digestibility, it resulted in a decreasing linear effect on ATT digestibility of crude protein (CP), fat, and TDF when compared to the control. It was concluded this was likely due to its poor fermentation characteristics in the colon. Short-chain fatty acids also were evaluated in this study at both the ileum and feces. There were no observed differences among the three treatments containing cellulose in terms of total ileal SCFA and the values were very low (avg. 2.1 m*M*). This was expected as there is limited fermentation in the small intestine. In terms of fecal acetate, isobutyrate, and butyrate concentrations, there were no statistical differences among treatments. Propionate concentrations tended to be higher in dogs fed the beet pulp treatment ($P < 0.10$) when compared to the low- and high-cellulose mixtures as well as the cellulose diet. Based on this study, ATT digestion of macronutrients was influenced by the presence of fiber as well as the levels of inclusion when compared to the control diet containing no added fiber.

Dietary Fiber in Feline Diets

Despite cats having no dietary requirements for carbohydrates, the importance of dietary fiber for intestinal and systemic health has sparked interest of the scientific community and pet food industry. Previous research has evaluated the effects of fiber fermentability on macronutrient digestibility, fecal characteristics, and postprandial metabolite responses in overweight cats (Fisher et al., 2012). In that study, the addition of fiber (i.e., beet pulp, wheat bran, or sugarcane fiber) decreased nutrient digestibility and energy content of the diets. Beet pulp was added at a 15.5% inclusion rate and was the most fermentable of the fiber sources tested, resulting in greater fecal concentrations of acetate, propionate, and lactate. The cats fed beet pulp also produced wetter feces with a lower pH, which was reflected in the fecal metabolite concentrations. Sunvold et al. (1995b) found that feline diets containing rapidly fermentable fibers (i.e., citrus pectin and carob bean gum) resulted in poor nutrient digestibilities, whereas the addition of moderately fermentable fibers (i.e., beet pulp) in cat diets could promote an optimal level of fermentative end-products (i.e., SCFA), which might maintain gastrointestinal tract health. Bueno et al. (2000a,b) found similar results. Twenty-eight cats were fed one of four diets; no added fiber, cellulose, beet pulp, and a pectin/gum arabic blend. The diets contained 3.0%, 8.8%, 8.4%, and 8.6% TDF, respectively. The pectin/gum arabic blend, which is a highly fermentable fiber, resulted in reduced food and water intake, and consequent body weight loss. The cats fed the pectin/gum arabic blend were noted to have loose and malodorous stools. It was also reported that cellulose provides an abrasive action that enhanced colonic weight and altered the mucosal morphology of the large intestine, while a highly fermentable fiber, such as the pectin/gum arabic blend, induces mucosal cell proliferation. In terms of SCFA production, cats fed the beet pulp treatment had the highest acetate and butyrate concentrations while pectin/gum arabic and no-fiber were intermediate, and cellulose had the lowest concentrations. There was no significant difference in propionate concentrations among treatments. From this study, beet pulp appeared to be the most effective dietary fiber when fed to cats at a moderate TDF level (approximately 8% DMB) compared to the other fiber treatments.

Barry et al. (2010) determined the effects of three fiber types on nutrient digestibility, fermentative end-products, and fecal microbial populations. Cellulose, fructooligosaccharides

(FOS), and pectin were added to the diets at a 4% inclusion rate and fed to twelve young adult male cats using a replicated 3 x 3 Latin square design. Within the three diets, the cellulose diet contained 7.9% TDF, the FOS diet contained 3.6% TDF, and the pectin diet had 6.7% TDF on a DMB. All of the diets had a greater amount of insoluble dietary fiber with the diets containing an insoluble to soluble ratio of 6.5:1.4, 3.6:2.6, and 6.7:4.6, respectively. However, the TDF value for the FOS diet was lower because the TDF method used could not quantify fructans, resulting in a lower reported TDF value. In terms of fecal characteristics, fecal pH was unaffected by treatment as was fecal output on a DM and as-is basis. In terms of ATT nutrient digestibility, DM and OM digestibilities did not differ among treatments. However, CP and acid hydrolyzed fat digestibilities tended to be lower in the pectin treatment (87.4 and 92.5%, respectively) when compared to the cellulose treatment (90.5 and 95.8%, respectively). Fecal scores and ammonia, 4-methyl phenol and BCFA concentrations were greater in both the FOS and pectin treatments. Indole concentrations were highest in the FOS treatment (2.4 µmole/g), followed by pectin (2.1 µmole/g), and cellulose (1.4 µmole/g). Although pectin-fed animals had higher amounts of phenols, indoles, and BCFA, which are seen as negative shifts in terms of fecal fermentative end-products, it also had higher SCFA concentrations when compared to the cellulose diet, balancing the pros and cons of the fiber source. Pectin feeding also resulted in the highest fecal butyrate concentration, which is a key energy source of colonocytes (Slavin, 2013). While the 4% inclusion level of highly fermentable fibers (i.e. FOS and pectin) increased SCFA concentrations, lower inclusion levels are utilized in practice and recommended in commercial diets (≤ 1%) to avoid unintended increases in fecal putrefactive compounds and poor stool quality.

SOYBEAN HULLS AS A DIETARY FIBER IN COMPANION ANIMAL NUTRITION

Soybeans are the second-most-planted field crop in the U. S., falling just behind corn. Soybean planting is very flexible and has steadily risen in yield improvements due to changes in seeding practices and low production costs (USDA, 2017). Additionally, price per bushel has decreased every year since 2010. The USDA estimated that the 2017-2018 soybean crop produced 4.4 billion bushels compared to the previous year of 4.3 billion bushels (Ash and Matias, 2018). One 60 lb bushel of soybeans produces an average of 11 lb of oil and 44 lb of meal (Blasi et al., 2000).

Soybean hulls are a co-product of the oil extraction process of the soybean seed and accounts for approximately 8% of the seed (Gnanasambandam and Proctor, 1999). Other co-products of the oil extraction process include soybean meal, soap stock, feed fat, and lecithin (Cherry, 2004). Each co-product is derived from a different stage in the extraction process and differs in nutrient composition (Blasi et al., 2000). However, except for soybean meal, many soy co-products are underutilized, including the hulls. The Association of American Feed Control Officials describes soybean hulls as consisting primarily of the outer covering of the soybean (AAFCO, 2017). Soybean hulls contain approximately 14-25% cellulose, 14-20% hemicelluloses, 10-12% pectin, 7-10% uronic acid, and 2-4% lignin (Mullin and Xu, 2001). Additionally, a large portion of the hull is composed of TDF. However, TDF content is variable as it has been reported to contain between 63 and 81% TDF (Cole et al., 1999).

The process to isolate the outer hull of the soybean consists of first drying and cracking the soybean seed with a roller to break it into smaller pieces. This helps facilitate the dehulling process. The hulls are removed via aspiration with the dehulled soybeans being further processed for oil extraction. The hulls are categorized into the following categories: large hulls and meats, small hulls and meats, and fines. The fines are returned to the primary soybean stream, whereas the soybean hull and meat fractions are further dehulled. Once the hulls are removed from the soybean meats and the meats are further processed to return to the soybean stream, the separated soybean hulls are toasted to destroy urease activity. Finally, they are then ground to the desired particle size and await packaging and shipment (Figure 1). The estimated yield of soybean hulls is approximately 5% of the original raw soybean weight (Blasi et al., 2000).

Cole et al. (1999) evaluated nine different sources of soybean hulls from various sources around the United States for nutrient content. Within the sources analyzed, TDF ranged from 63.8% to 81.2% and the ratio of insoluble to soluble fiber ranged from 5.0:1 to 15.4:1, which indicates wide variability. Additionally, this paper evaluated one of the soybean hull sources (76.4% and 5.0:1 TDF and insoluble:soluble ratio, respectively) at varying dietary inclusion levels using thirty adult beagles. All of the diets were formulated to be isonitrogenous and mirror a premium dog diet. The experimental diets contained 0, 3.0, 4.5, 6.0, 7.5, and 9.0% soybean hulls corresponding to 0.4, 3.0, 4.1, 6.0, 7.3, and 8.9% TDF on DMB in the diet, respectively (n=5). As soybean hull inclusion levels increased, DM, OM, and GE digestibility decreased. Additionally, TDF digestibility increased with increasing levels of soybean hulls. Based on these results, the authors compared the diets containing 0, 6.0, 7.5, and 9.0% soybean hulls with a 7.5% beet pulp control diet using ileal cannulated dogs to determine the optimal level of soybean hull inclusion relative to a beet pulp control diet. The inclusion of supplemental fiber did not affect nutrient digestion at the ileum. However, ATT digestibility of DM and OM were lower in dogs fed 6.0, 7.5, and 9.0% soybean hulls compared to the 0% fiber diet and the beet pulp control diet. When comparing the beet pulp and soybean hull diets, there were no differences in nutrient digestibility. This indicates that soybean hulls behaved similarly to beet pulp in terms of ATT nutrient digestibility. However, fecal fermentative end-products were not measured, which would have been beneficial to understand the impact of soybean hulls on parameters related to gastrointestinal health.

Burkhalter et al. (2001) evaluated soybean hulls containing varying insoluble to soluble fiber ratios. Seven different diets were fed to six ileal cannulated dogs in a 6 x 7 Youden square design. The treatments included no supplemental fiber, beet pulp, and five soybean hull diets containing an insoluble to soluble (I:S) ratio of 1.9, 2.7, 3.2, 5.2, and 7.2. The different ratios were achieved by obtaining five different samples of soybean hulls from four different U.S. processing plants, indicating that soybean hulls can vary among sources and based on processing parameters. Ileal digestibility of DM, OM, CP, TDF, fat, and GE were lower in all of the fiber-containing diets compared to the no-fiber diet. In terms of ATT digestibility, added fiber had a negative effect on DM (69.2-75.0%), OM (77.0-82.1%), fat (91.5-94.0%), and GE (80.3-83.8%) digestibilities when compared to the no fiber diet (78.5, 85.7, 94.0, and 86.1%, respectively). As expected, the no fiber diet had the lowest fecal output and, as the amount of fiber increased, as-is fecal output increased as well. The beet pulp diet resulted in higher moisture content in the feces, indicating a greater water holding capacity. Overall, the diets containing an I:S ratio lower than 2.0 or higher than 5.0 minimized the negative effects on ileal nutrient digestibility. However, within all of the I:S soybean hull ratios, the highest TDF value was 8.6% and the lowest was 7.4%, which corresponded to soybean hull diets containing an I:S ratio the 3.2, 1.9 and 5.2, respectively. There is still a lack of literature utilizing soybean hulls as the main source of dietary fiber in canine diets with a TDF content above 10%.

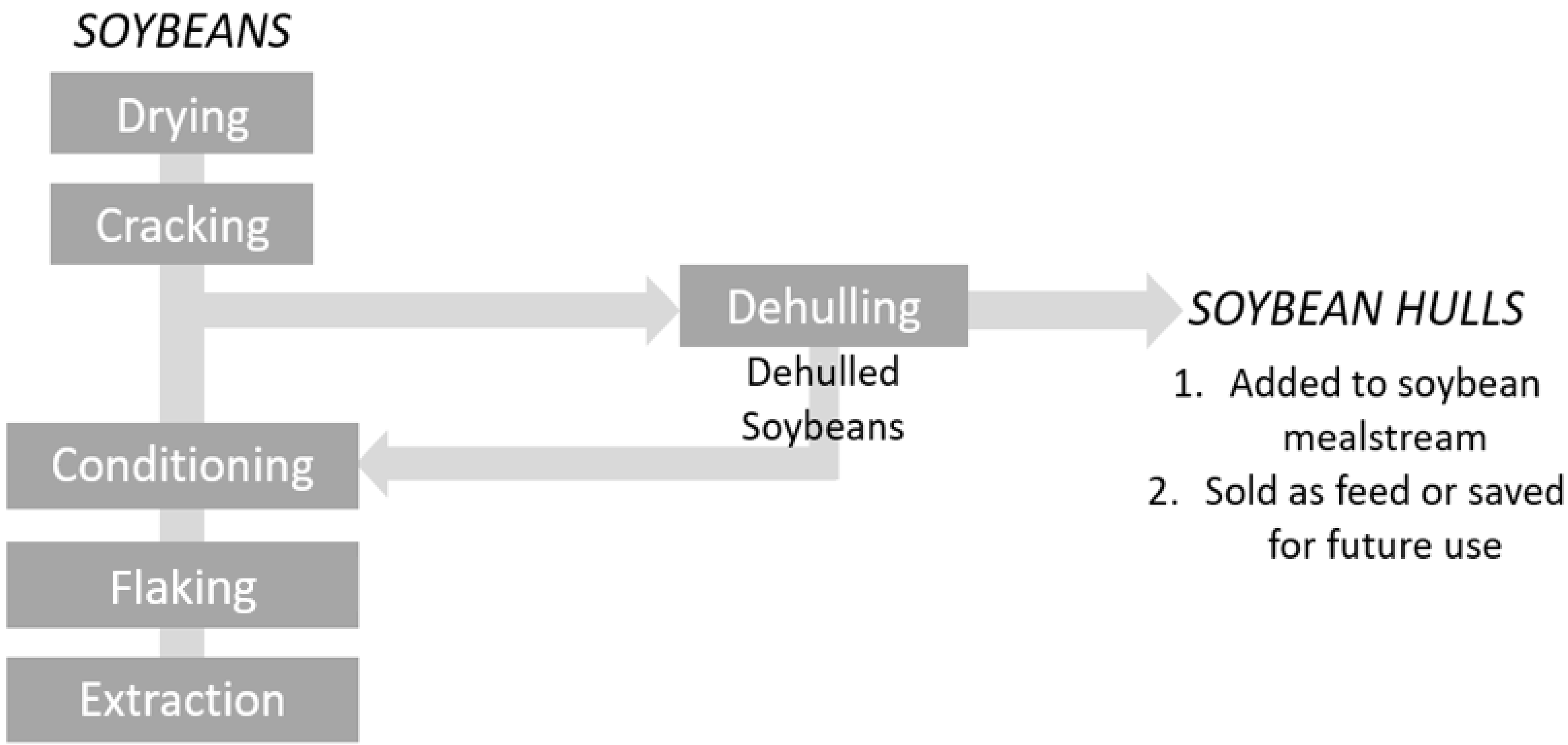
Previous research evaluating soybean hulls has been done with canines, however there is a lack of research evaluating soybean hulls in feline diets. Nevertheless, there has been a recent increased emphasis on health and wellbeing of companion dogs and cats, as well as an increased interest in gaining a deeper understanding of the effects of dietary fiber in these animal species. To achieve this, the portfolio of fiber sources that can be successfully used in pet foods needs to broaden. Soybean hulls are an economical and readily available product and have yet to be explored in monogastric nutrition at high inclusion levels (approximately 15% TDF). The effects of soybean hulls on gastrointestinal health and nutrient digestibilities by dogs and cats should be examined to verify if it performs similarly to common fiber sources used in pet foods.

THESIS OBJECTIVES AND HYPOTHESIS

Due to the limited number of literature evaluating soybean hulls in canine and feline diets, the objective of this thesis was to evaluate the effects of soybean hulls on outcomes related to gastrointestinal function and health in comparison to no fiber, beet pulp, and cellulose diets. Two studies were conducted to evaluate the effect of soybean hulls on food intake, apparent total tract nutrient digestibilities, fermentative end-products, and fecal quality when fed to dogs and cats. It was hypothesized that soybean hull intake would beneficially shift fecal fermentative end-products without compromising nutrient utilization and animal health and exhibiting an intermediate fermentative profile in comparison to beep pulp and cellulose.

FIGURE

Figure 2.1. Soybean hull processing schematic adapted from Blasi et al. (2000).



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CHAPTER 3:

EFFECTS OF HIGH INCLUSION OF SOYBEAN HULLS ON APPARENT TOTAL TRACT MACRONUTRIENT DIGESTIBILITY, FECAL QUALITY, AND FECAL FERMENTATIVE END-PRODUCT CONCENTRATIONS IN EXTRUDED DIETS OF ADULT DOGS

ABSTRACT

Soybean hulls (**SBH**) are a fiber-rich co-product of the soybean oil extraction process that corresponds to 8% of the soybean seed. Despite being readily available and priced competitively, SBH are underutilized in monogastric nutrition. Thus, the objective of this study was to evaluate the use of SBH as a dietary fiber in canine diets. Four diets were formulated with either SBH, beet pulp (**BP**), or cellulose (**CL**) as the main source of dietary fiber (15% total dietary fiber [**TDF**]), with the control diet formulated with no supplemental fiber (**NF**). All animal procedures were approved by the University of Illinois Institutional Animal Care and Use Committee. Eight adult female beagles (mean age = 4.6 ± 0.6 yr; mean BW = 12.8 ± 1.7 kg) were used in a replicated 4x4 Latin square design. Each period consisted of 14 d, with 10 d of diet adaptation followed by 4 d of total fecal and urine collections. At the end of each period, a blood sample was collected and analyzed for serum chemistry. Food was offered twice daily and fed to maintain body weight. Food intake (g/d) on a dry matter basis (**DMB**) did not differ among treatments. Fecal score was lower ($P < 0.05$) for dogs fed CL (2.0) in contrast with other dietary treatments (2.3), using a 5-point scale. Fecal as-is and DM output did not differ for dogs fed BP, CL, or SBH, and were approximately 50% higher ($P < 0.05$) than dogs fed NF. Apparent total tract (**ATT**) dry matter, organic matter, and gross energy digestibilities were greater ($P < 0.05$) for dogs fed NF when compared to dogs fed BP, CL, or SBH. Dogs fed CL had greater ($P < 0.05$) ATT fat digestibility (94%) compared with all other treatments (mean = 91%). Dogs fed CL and NF had greater ($P <$ 0.05) ATT crude protein digestibility, 87% and 86%, respectively, while dogs fed SBH were intermediate (83%) and dogs fed BP were lowest (79%). Total short-chain fatty acid (**SCFA**) concentration was greatest in dogs fed BP (582.5 μmole/g) and SBH (479.7 μmole/g) when compared to NF and CL (267.0 and 251.1 μmole/g, respectively). Serum metabolites were within reference ranges and animals remained healthy throughout the study. In conclusion, SBH resulted in similar ATT macronutrient digestibilities when compared to BP and CL. Dogs fed SBH also were observed to have an increase in fecal SCFA. In general, high level addition of SBH were well-utilized by the dog, resulting in no untoward effects on dog health, nutrient digestibility, or fecal characteristics.

INTRODUCTION

The most recent pet census, published in 2018, indicates that there are approximately 89.7 million pet dogs in the United States (APPA, 2018). Due to pet parents acknowledging their pet as a family member, owners have developed a strong emotional bond with their pets and seek ways to increase their health and longevity. Dietary fibers have gained renewed interest in the pet food industry as a functional ingredient, as they have several health benefits such as control of glucose homeostasis, lowering caloric density of foods and aiding in weight loss, improving digestive health, improving fecal quality, and increasing production of short-chain fatty acids (**SCFA**), which are used as an energy source for the colonocytes and host (Swanson et al., 2002; Fahey et al., 1992; Massimino et al., 1998; Banta et al., 1979; den Besten et al., 2013).

Soybean hulls (**SBH**) are a fiber-rich co-product of the soybean oil extraction process. In the U.S., soybean production is increasing, and it is followed by a steady decrease in yearly costs (USDA, 2017), resulting in copious amounts of soybean co-products being readily available.

However, no research has been done to evaluate the effects of SBH in canine diets with high concentrations of dietary fiber. Hence, the objective of this study was to evaluate the effects of SBH on food intake, apparent total tract macronutrient digestibility, and fecal fermentative end-products of dogs compared to a no fiber diet (control) and two standard fibers - beet pulp and cellulose. It was hypothesized that intake of SBH would beneficially shift fermentative end-product production without compromising nutrient digestibility and animal health.

MATERIALS AND METHODS

All animal care procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to animal experimentation.

Animals and Diets

Eight adult female beagles (mean age = 4.6 yr; mean body weight = 12.8 ± 1.7 kg) were used. The dogs were housed in a temperature- and light-controlled room (14 h light: 10 h dark) at the Veterinary Medicine Basic Sciences building at the University of Illinois at Urbana-Champaign. Dogs were housed individually in pens (1.2 m wide x 1.8 m long) and socialized in groups with toy enrichment. Pens allowed for nose-nose contact between dogs in adjacent runs and visual contact with all dogs in the room. Water was available ad libitum throughout the study.

Four experimental diets were fed to the dogs twice daily (0800 and 1600) to maintain body weight (**BW**). Food intake and refusal were recorded after each meal throughout the duration of the study. Food intake was determined based on previous individualized food intake and metabolizable energy (**ME**) requirement records. The control diet contained no supplemental dietary fiber (no fiber; **NF**), while the three fiber diets were formulated with either beet pulp

(**BP**), cellulose (**CL**), or SBH as the main source of dietary fiber added at the expense of chicken by-product meal and brewer's rice (**Table 3.1**). The diets were formulated to be complete and balanced according to AAFCO (2016) for adult dogs at maintenance, and to have a similar nutrient composition (**Table 3.2**). Macronutrients were targeted to contain approximately 30% crude protein (**CP**), 12% acid hydrolyzed fat (**AHF**), and 15% total dietary fiber (**TDF**), except for the NF diet (5% TDF). Diets were extruded at the Kansas State University Bioprocessing and Industrial Value-Added Program facility (Manhattan, KS).

Experimental Design and Sample Collection

This experiment followed a replicated 4x4 Latin square design so each animal served as its own control. Each experimental period consisted of 14 d. The first 10 d served as the diet adaption phase, followed by 4 d of total fecal and urine collection. Throughout the collection phase, all feces were collected, scored using the following 5-point scale: 1= hard, dry pellets; small hard mass; 2 = hard formed, remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured. All samples were stored in a -20℃ freezer until analysis. Within the collection period, one fresh fecal sample was collected from each dog within 15 min of defecation. The fresh sample was scored, weighed, and measured for pH. Aliquots of the sample were frozen immediately at -20℃ to be used to determine phenol and indole concentrations. Additionally, an additional aliquot was placed in 2 N hydrochloric acid and frozen at -20°C until analysis of SCFA, branched-chain fatty acids (**BCFA**), and ammonia concentrations. Total urine output was collected from d 11-14 in containers containing 5 mL 2 N hydrochloric acid for immediate acidification upon urination. Acidified urine samples were subsampled and stored at -20°C until analysis. Additionally, at the end of every period, an 8 mL fasted blood sample from each dog was collected. Blood was collected in BD Vacutainer serum separator tubes and EDTA tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) for serum chemistry and complete blood count, respectively. All samples were analyzed by the University of Illinois Veterinary School Diagnostics Laboratory using a Hitachi 911 clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN).

Sample Preparation and Chemical Analyses

Food and fecal samples were used to determine apparent total tract (**ATT**) macronutrient digestibility. Samples were dried at 55°C in a forced-air oven and ground in a Wiley mill (model 4; Thomas Scientific, Swedesboro, NJ) through a 2-mm screen. Diet and fecal samples were analyzed for dry matter (**DM**), organic matter (**OM**), and ash according to AOAC (2006; methods 934.01 and 942.05). Crude protein content of the diets and fecal samples was calculated from Leco (TruMac N, Leco Corporation, St. Joseph, MI) total nitrogen values according to AOAC (2006; method 992.15). Total lipid content was determined by acid hydrolysis followed by ether extraction according to the methods of the American Association of Cereal Chemists (1983) and Budde (1952). Diet and fecal TDF content were analyzed by Eurofins (Des Moines, IA) according to AOAC (1995, Method 991.43). Diet, fecal, and urine samples were measured for gross energy (**GE**) by bomb calorimeter (Model 6200, Parr Instruments Co., Moline, IL). Urine GE values were used to calculate ME.

Fecal samples were analyzed for phenol and indole concentrations using gas chromatography according to the method of Flickinger et al. (2003); SCFA and BCFA concentrations using gas chromatography according to Erwin et al. (1961); and ammonia concentrations were measured according to the method of Chaney and Marbach (1962).

Statistical Analyses

All data were analyzed using the Mixed Models procedure of SAS (SAS Institute Inc., version® 9.4, Cary, NC), with diet as a fixed effect and dog as the random effect. Fecal score was analyzed using the GLIMMIX procedure of SAS. Data normality was analyzed using PROC UNIVARIATE. Differences among treatments were determined using a Fisher-protected least significant difference test with a Tukey adjustment to control for type-1 experiment-wise error. A probability of $P \leq 0.05$ was accepted as statistically significant and reported pooled standard errors of the mean (**SEM**) were determined according to the Mixed Models procedure of SAS.

RESULTS AND DISCUSSION

Diet, Food Intake, and Fecal Characteristics

All four experimental diets had relatively similar chemical composition (**Table 3.2**). Dry matter content ranged from 90.2 to 95.8%. On a DM basis, OM among all experimental diets was approximately 93% and average crude protein was 31.7%. The CL treatment had slightly higher AHF content (15.9%) in contrast with NF (11.9%), BP (13.0%), and SBH (13.3%), which was reflected in the higher GE content of the CL diet. The variation observed in the AHF content of these diets was due to deviations in the amount of fat dispersed on the kibbles during the fat coating step during diet manufacturing. The NF diet was formulated to have 5.0% TDF and the fiber treatments to have 15% TDF. This was achieved for the most part. The BP diet had a slightly higher amount of TDF (17.3%) than expected. This result is not surprising as TDF content of BP can be highly variable (Fahey et al., 1990; Sunvold et al., 1995). As intended, the soluble and insoluble fractions of TDF varied among the dietary treatments, with BP diet having a greater concentration of soluble fiber (7.2%) in comparison with CL (2.6%) or SBH (1.9%) diets.

Food intake (g/d DM basis) did not differ ($P > 0.05$) among the four dietary treatments (**Table 3.3**). Dogs fed the CL treatment had a lower ($P < 0.05$) fecal score (2.0) compared to the other three treatments (2.3). All of these scores, however, were within an acceptable range using a 5-point scale. In addition, similar fecal score (2.12) has been reported for dogs fed a diet formulated with 12% BP and containing 13% TDF (Kroger et al., 2017). Fecal as-is and DM output (g/d) did not differ ($P > 0.05$) in dogs fed BP, CL, and SBH, however they were greater than dogs fed the NF diet (**Table 3.3**). Numerically, BP had the highest fecal output expressed on an as-is basis (85.5 g/d) compared to CL, and SBH (66.0 and 69.2 g/d, respectively). But, when expressed on a DM basis, BP fecal output (27.9 g/d) was no longer the highest, being similar to CL and SBH (31.6, and 27.2 g/d). This indicates that the BP diet had a greater water holding capacity due to the higher concentration of soluble fibers. This property has also been previously reported in sows fed diets containing 14% BP and canines fed diets containing 7.5% BP as the main source of dietary fiber (Burkhalter et al., 2001; Serena et al., 2008).

Apparent Total Tract Macronutrient and Energy Digestibility

Apparent total tract digestibility coefficients for dogs fed the experimental diets are presented in **Table 3.4**. Dogs fed BP, CL, and SBH had lower ($P < 0.05$) ATT DM (76.2, 77.2, and 79.6%, respectively) and OM digestibilities (80.9, 80.5, and 79.9%, respectively) when compared to the NF diet (DM: 85.4 and OM: 90.1%). Fahey et al. (1990) reported a linear decrease in DM and OM digestibilities as BP inclusion increased from 0-12.5% in diets for adult dogs. In that study, the highest inclusion of BP (12.5%) amounted to 13.7% TDF, and DM and OM digestibilities of 84.3 and 87.6%, respectively, were noted. More recently, Kroger et al.

(2017) also reported an OM digestibility of 86.7% in dogs fed a diet containing 12% BP and 13.1% TDF. Middelbos et al. (2007) noted ATT DM and OM digestibilities of 85.4 and 83.2%, and 91.0 and 88.7%, respectively, for diets containing either 2.5% BP or CL. Silvio et al. (2000) reported DM digestibility of 81.3% in dogs fed diets containing 10% CL. The lower coefficients for DM and OM observed in the current study might be attributed to the higher inclusion levels of BP and, consequently, greater TDF concentration (17.3%). It is known that dietary fiber concentration may have a negative correlation with coefficients of ATT nutrient digestibility (Kienzle et al., 2001; Davison and McDonald, 1998).

Dogs fed the NF and CL treatments had the greatest ($P < 0.05$) ATT CP digestibility (85.8% and 87.1%, respectively), followed by the SBH diet (83.3%), with BP having the lowest value (78.8%). Muir et al. (1996) reported similar CP digestibility by dogs fed CL (86.7%). Although CP digestibility was lowest in dogs fed BP, BP is a fermentable fiber due to its higher soluble fiber content. This undoubtedly resulted in more microbial growth leading to more microbial N to be excreted in the feces, giving a false sense of undigested protein and resulting in lower apparent CP digestibility as suggested by Sunvold et al. (1995). Additionally, dietary fiber also may have an abrasive effect on the gastrointestinal tract increasing the elimination of sloughed cells in the feces and, as such, resulting in greater loss of endogenous proteins and lower ATT CP digestibility (Wilfart et al., 2007). In contrast to our findings, Kroger et al. (2017) observed slightly higher CP digestibility by dogs fed BP (82.5%); however, the diet fed had a lower TDF content (13%). Cole et al. (1999), however, observed similar CP digestibility (83.2%) by dogs fed a SBH diet containing 8.9% TDF. This suggests that CP digestibility could stabilize once SBH inclusion reaches a certain inclusion level.

Dogs fed the CL treatment had greater (*P* < 0.05) ATT AHF digestibility (94.3%) than those fed NF, BP, and SBH treatments (90.9, 91.2, and 91.9%, respectively). The greater AHF digestibility observed in dogs fed CL diet could be related to the higher concentration of AHF fat in this diet. Our findings are in contrast with previous literature supporting higher AHF digestibility (93.7-97.7%) in adult dogs fed diets containing moderate or high levels of BP (Fahey et al., 1990; Fahey et al., 1992; Kroger et al., 2017). Despite the greater concentration of TDF in the experimental diets in the current study, the source of fat used herein (i.e., choice white grease) also differed from most of the previous studies (e.g., chicken fat or vegetable oil). Thus, it is possible that not only the amount, but also the type of dietary fat affected our results. Previous research in puppies found that fat digestibility of beef tallow was dependent upon the unsaturated fatty acid concentration of the diet, with greater ratios of unsaturated to saturated fatty acids, resulting in a positive effect on digestibility (Meyer et al., 1992). Marx et al. (2015) also demonstrated that diets containing unsaturated fat sources had greater fat digestibility in dogs. For comparison, choice white grease has a unsaturated:saturated ratio of 0.31, in contrast to 0.71 for poultry fat, and 4.07 for soybean oil (NRC, 2006).

Total dietary fiber digestibility was highest in dogs fed the BP treatment (48.2%), while NF was intermediate (37.8%), and CL and SBH had the lowest ATT digestibilities of TDF; 15.1% and 22.7%, respectively. These are expected results because CL and SBH have a higher ratio of insoluble to soluble fiber (12.1:2.6 and 12.4:1.9). Slightly higher TDF digestibility was observed in dogs fed SBH at a 9% inclusion (28.5%) and BP at a 12.5% inclusion level (57.5%) (Cole et al., 1999; Fahey et al., 1990). Higher TDF digestibility was also observed by Fahey et al. (1992) when dogs were fed 7.5% BP (61.1%). The discrepancy among these findings could be possibility due to variations in the fiber composition of different sources of SBH and BP. The

TDF content of the SBH ingredient fed in the current study was 80.7% TDF and an insoluble to soluble fiber ratio of 7:1. Previous research evaluated SBH TDF content of various sources that ranged from 63.8% to 81.2% and the ratio of insoluble to soluble fiber ranged from 5.0:1 to 15.4:1 (Cole et al., 1999). Insoluble fibers are less fermentable in the large intestine of monogastric animals, resulting in most of it being excreted in the feces. Soluble fibers are more easily fermented by microbes that harbor in the distal portions of the gastrointestinal tract. Thus, greater proportions of soluble, fermentable fibers will be degraded, resulting in subsequent greater ATT TDF digestibility.

Digestible and metabolizable energy digestibilities were similar for dogs fed the three fiber-supplemented diets, but lower ($P < 0.05$) than for dogs fed the NF diet. Added dietary fiber dilutes the caloric density and may decrease nutrient digestibility (Weber et al., 2007), which is reflected in the digestible energy (**DE**) and ME values for the fiber treatments. Cole et al. (1999) reported a slightly higher DE digestibility value for dogs fed 9% SBH (87.4%). Additionally, Fahey et al. (1992) observed higher DE and ME digestibility values for dogs fed 7.5% BP (90.2 and 87.9%, respectively). However, the diets in the mentioned literature did not approach the high TDF content of our experimental diets, which could result in the differences in DE and ME digestibilities. However, this lower digestibility content is not a negative attribute. Higher inclusion levels of dietary fibers may improve satiety and have weight management applications, as suggested by Weber et al. (2007). With the continued increase in pet obesity, SBH may become a sustainable functional ingredient in diets targeting weight loss or management. However, future studies are warranted in this area.

Fecal Fermentative End-Products and Serum Chemistry

Fecal fermentative end-products were affected by the different fiber sources added in the experimental diets (**Table 3.5**). It is assumed that the higher production of fermentative end-products measured in the feces is reflective of an augmented colonic fermentative process in the dog. Total SCFA concentration was greatest (µmole/g, DMB) for dogs fed BP and SBH, indicating that there was increased saccharolytic fermentation occurring. Dogs fed NF and CL had lower ($P < 0.05$) total SCFA concentrations. Bosch et al. (2009) reported similar total SCFA production values in dogs fed a highly fermentable diet that contained 8.5% BP (540 µmole/g DMB) or a low fermentable diet that contained 8.5% CL (260 µmole/g DMB). Zentek (1996), however, observed lower total SCFA production (194 µmole/g) when feeding dogs a CL diet with similar TDF content (13.7%). Dogs fed BP and SBH had greater ($P < 0.05$) acetate and propionate concentrations than dogs fed the NF and CL treatments. Kroger et al. (2017) reported slightly lower values for acetate (214 µmole/g) and propionate (64.8 µmole/g) in dogs fed diets containing 12% BP (13.1% TDF). Lower acetate (127 and 276 µmole/g) and propionate (49 and 93 µmole/g) concentrations were also observed by Middelbos et al. (2007) in dogs fed CL and BP diets. However, CL and BP were only added at a 2.5% inclusion level in the previously mentioned study (5.07 and 4.03% TDF, respectively). Dogs fed the BP treatment had the greatest ($P < 0.05$) butyrate concentration (45.5 µmole/g), while NF and SBH had intermediate concentration (33.5 and 37.7 µmole/g, respectively), and CL having the lowest value (23.8 µmole/g). Butyrate is preferentially taken up by the colonocytes compared with acetate and propionate. Butyrate provides energy to the colonocytes as well as having potential protective qualities against diseases, such as ulcerative colitis as observed in humans (Christl et al., 1996). Not only did BP and SBH result in greater amounts of fecal SCFA, they resulted in similar proportions of acetate (70.7 and 66.9%), propionate (21.5 and 25.2%), and butyrate (7.8 and 7.9%) of the total SCFA produced.

Total phenol and indole concentrations (μmole/g, DMB) were greatest ($P < 0.05$) in dogs fed NF and CL treatments (3.1 and 2.2 μmole/g, respectively). Intermediate concentrations (1.7 μmole/g) resulted from SBH feeding, whereas BP had the lowest concentration of total phenols and indoles (0.9 μmole/g). The highest concentrations of ammonia were observed in dogs fed the NF and SBH treatments (152.2 and 147.8 μmole/g), and lowest (103.9 μmole/g) for dogs fed BP, whereas dogs fed the CL treatment had similar ammonia concentration (129.3 μmole/g) when compared with all other treatments. BP and SBH had the lowest fecal pH, which has been seen to reduce the resorption of ammonia (Matsuoka et al., 1990).

Branched-chain fatty acids are putrefactive compounds that result undesirable fecal characteristics. They also are produced when energy is limited in the colon (Middelbos et al., 2007). Total BCFA concentrations were significantly greater ($P < 0.05$) in dogs fed the NF treatment compared to the three fiber treatments, possibly indicating limited energy available in the large intestine. Dogs fed NF had the greatest ($P < 0.05$) isobutyrate concentrations (8.8 μmole/g), whereas CL was observed to have intermediate concentrations (6.6 μmole/g), and dogs fed BP and SBH contained the lowest isobutyrate concentrations (4.7 and 6.3 μmole/g, respectively). NF also had the greatest ($P < 0.05$) isovalerate concentration (13.3 μmole/g), while CL and SBH had intermediate concentrations (both containing 9.8 μmole/g), and BP had the lowest concentration (6.1 μmole/g). Dogs fed BP had the greatest ($P < 0.05$) valerate concentration (1.3 μmole/g), while no differences were observed among dogs fed NF, CL, and SBH diets (0.8, 0.7, and 0.9 μmole/g). Kroger et al. (2017) observed similar isovalerate (6.0

μmole/g), isobutyrate (4.5 μmole/g), and valerate (0.5 μmole/g) concentrations in dogs fed 12% BP.

Phenols, indoles, ammonia, and BCFA are putrefactive compounds that result from bacterial fermentation in the hindgut and causes foul-smelling feces, which can be an unappealing quality to a diet from the pet owner's standpoint (Miner and Hazen, 1969; O'Neill and Phillips, 1992). The amount of putrefactive compounds that are produced depends on the amount and type of fiber present in the diet (Vince et al., 1990; Flickinger et al., 2003). Increased amounts of rapidly fermentable fibers, such as pectins, have been observed to cause an increase in peptides and amino acids produced in the proximal colon, followed by microbes fermenting these substrates leading to the formation of these undesirable putrefactive compounds (Barry et al., 2010; Kanakupt et al., 2011). Low concentrations of these putrefactive compounds were observed in dogs fed SBH in the current study. These values mirrored dogs fed BP, thus highlighting the benefits of SBH as a dietary fiber source.

Serum chemistry and complete blood count were analyzed as part of this for each dog to ensure that the experimental diets did not have any negative health implications. Serum metabolite data are presented in **Table 3.6**. There were no differences among treatments and all values were within the corresponding reference ranges. A previous study done by Scheraiber et al. (2016) evaluated blood biochemical profile of dogs fed a diet with 16% SBH (25% TDF) or without SBH (control diet, 14% TDF). Similar to our findings, the authors did not observe differences between dietary treatments for cholesterol, triglycerides, or glucose. Likewise, those metabolites were within reference ranges for healthy adult dogs. In the study presented herein, complete blood count were also analyzed and deemed normal for adult healthy dogs (data not shown).

IMPLICATIONS

Based on the results of this study, dogs fed SBH as a dietary fiber source had similar ATT macronutrient digestibilities when compared to the other two fiber treatments, BP and CL. Dogs tolerated the SBH dietary treatment well, and there were no detrimental effects on fecal quality or health status. Additionally, dogs fed SBH had a beneficial shift in fecal fermentative end-products when compared to the CL and NF diets; SCFA concentrations increased while phenols, indoles, and BCFA concentrations decreased. Although the SBH diet had a higher insoluble:soluble fiber ratio than CL, the results of this study indicated that the SBH diet was comparable to the BP diet, suggesting that the insoluble fraction of SBH might be more fermentable than CL. Based on the findings of this research, SBH is a suitable dietary fiber source in canine diets when fed at high concentrations of approximately 15% TDF. As such, SBH can be used as an economical, readily available, and sustainable dietary fiber source in pet food formulations for adult dogs. Soybean hulls also has the potential to be a functional ingredient in diets for dogs, modulating gastrointestinal health and aiding in weight management. Further studies should be conducted to confirm this hypothesis.

TABLES

Table 3.1. Ingredient composition of experimental diets containing select fiber sources and fed to dogs.

	Treatments			
Item, % as-is basis	**No Fiber**	**Beet Pulp**	**Cellulose**	**Soybean Hulls**
Chicken by-product meal	31.1	31.7	33.3	30.7
Corn gluten meal	8.2	8.2	8.2	8.2
Brewer's rice	46.0	28.8	33.5	31.5
Corn, whole, ground	4.1	4.1	4.1	4.1
Choice white grease	8.0	8.0	8.0	8.0
Beet pulp[1]	0.0	16.6	0.0	0.0
Cellulose[1]	0.0	0.0	10.3	0.0
Soybean hulls[1]	0.0	0.0	0.0	15.0
Salt	0.5	0.5	0.5	0.5
Potassium chloride	0.45	0.45	0.45	0.45
Taurine	0.2	0.2	0.2	0.2
Mineral premix[2]	0.18	0.18	0.18	0.18
Vitamin premix[3]	0.18	0.18	0.18	0.18

[1]Lortscher Animal Nutrition Inc., Bern, KS.
[2]Provided per kg diet: 17.4 mg manganese ($MnSO_4$), 284.3 mg iron ($FeSO_4$), 17.2 mg copper ($CuSO_4$), 2.2 mg cobalt ($CoSO_4$), 166.3 mg zinc ($ZnSO_4$), 7.5 mg iodine (KI), and 0.2 mg selenium (Na_2SeO_3).
[3]Provided per kg diet: 11,000 IU vitamin A Acetate; 900 IU vitamin D3; 57.5 IU vitamin E Acetate; 0.6 mg vitamin K; 7.6 mg thiamine HCl; 11.9 mg riboflavin; 18.5 mg pantothenic acid, d, calcium; 93.2 mg niacin; 6.6 mg pyridoxine HCl; 12.4 mg biotin; 1,142.1 µg folic acid; 164.9 µg vitamin B12, 0.1% mannitol.

Table 3.2. Analyzed chemical composition and energy content of experimental diets.

Item	Treatments			
	No Fiber	**Beet Pulp**	**Cellulose**	**Soybean Hulls**
Dry matter (%)	90.2	93.3	95.8	94.1
	---------- %, DM basis ----------			
Organic matter (%)	93.1	92.7	93.2	92.9
Crude protein (%)	31.7	31.4	32.6	30.9
Acid-hydrolyzed fat (%)	11.9	13.0	15.9	13.3
TDF[1] (%)	5.0	17.3	14.7	14.3
Insoluble (%)	2.4	10.1	12.1	12.4
Soluble (%)	2.6	7.2	2.6	1.9
GE[1] (kcal/g; measured)	5.0	5.0	5.2	5.0
ME[2] (kcal/g; calculated)	3.7	3.3	3.5	3.4

[1]TDF = total dietary fiber; GE = gross energy (measured by bomb calorimetry).
[2]ME= metabolizable energy; ME = 8.5 kcal ME/g fat + 3.5 kcal ME/g CP + 3.5 kcal ME/g nitrogen-free extract.

Table 3.3. Food intake and fecal characteristics of dogs fed diets containing select fiber sources.

Item	Treatments				SEM	*P*-value
	No Fiber	Beet Pulp	Cellulose	Soybean Hulls		
Food intake						
g/d, DM basis	112.5	117.5	140.5	116.3	17.64	0.2218
Fecal characteristics/output						
Fecal score[1]	2.3[a]	2.3[a]	2.0[b]	2.3[a]	0.08	0.0002
Fecal output, as-is (g/d)	35.9[b]	85.5[a]	66.0[a]	69.2[a]	11.01	0.0001
Fecal output, DMB (g/d)	15.9[b]	27.9[a]	31.6[a]	27.2[a]	3.85	0.0002
Fecal DM (%)	44.7[a]	33.8[c]	48.7[a]	39.4[b]	1.68	0.0001

[1]Fecal scores: 1= hard, dry pellets; small hard mass; 2 = hard formed, remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured.

[a-b] Means in the same row without common superscript letters denote a significant difference ($P < 0.05$).

Table 3.4. Total tract apparent macronutrient and energy digestibilities of dogs fed diets containing different fiber sources.

	Treatments					
Item	**No Fiber**	**Beet Pulp**	**Cellulose**	**Soybean Hulls**	**SEM**	***P*-value**
Nutrient and energy digestibilities, %						
Dry matter	85.4[a]	76.2[b]	77.2[b]	79.6[b]	0.82	0.0001
		---------- %, DM basis ----------				
Organic matter	90.1[a]	80.9[b]	80.5[b]	79.9[b]	0.73	0.0001
Crude protein	85.8[a]	78.8[c]	87.1[a]	83.3[b]	0.82	0.0001
Acid-hydrolyzed fat	90.9[b]	91.2[b]	94.3[a]	91.9[b]	0.62	0.0002
TDF[1]	37.8[a]	48.2[a]	15.1[b]	22.7[b]	2.91	0.0001
DE[1]	89.0[a]	81.3[b]	82.8[b]	81.2[b]	0.75	0.0001
ME[1]	83.4[a]	75.0[b]	77.4[b]	74.8[b]	1.25	0.0001

[1]TDF = total dietary fiber; DE = digestible energy; ME = metabolizable energy.
[a-c] Means in the same row without common superscript letters denote a significant difference ($P < 0.05$).

Table 3.5. Fecal fermentative end-products for dogs fed diets containing select fiber sources.

Item (µmole/g DM basis)	Treatments				SEM	*P*-value
	No Fiber	Beet Pulp	Cellulose	Soybean Hulls		
Fecal pH	6.3[a]	5.9[b]	6.6[a]	5.9[b]	0.10	0.0001
Ammonia	152.2[a]	103.9[b]	129.3[ab]	147.8[a]	11.53	0.0021
Phenols & Indoles						
Total Phenols/Indoles	3.1[a]	0.9[b]	2.2[a]	1.7[ab]	0.48	0.0021
Phenol	0.9	0.1	0.7	0.3	0.29	0.0862
Indole	2.2[a]	0.8[b]	1.5[ab]	1.4[ab]	0.25	0.0019
SCFA[1]						
Total SCFA[1]	267.0[b]	582.5[a]	251.1[b]	479.7[a]	41.43	0.0001
Acetate	150.8[b]	411.9[a]	156.9[b]	321.0[a]	28.48	0.0001
Propionate	82.6[b]	125.1[a]	70.3[b]	121.0[a]	10.56	0.0003
Butyrate	33.5[ab]	45.5[a]	23.8[b]	37.7[ab]	4.91	0.0037
BCFA[1]						
Total BCFA[1]	22.9[a]	12.1[b]	17.1[b]	17.0[b]	1.51	0.0003
Isobutyrate	8.8[a]	4.7[b]	6.6[ab]	6.3[b]	0.59	0.0009
Isovalerate	13.3[a]	6.1[c]	9.8[b]	9.8[b]	0.97	0.0001
Valerate	0.8[ab]	1.3[a]	0.7[b]	0.9[ab]	0.13	0.0121

[1]SCFA = short chain fatty acids; BCFA = branched chain fatty acids.

[a-c] Means in the same row without common superscript letters denote a significant difference ($P < 0.05$).

Table 3.6. Serum metabolites for dogs fed diets containing select fiber sources.

Item	Reference Range[1]	No Fiber	Beet Pulp	Cellulose	Soybean Hulls	SEM
		Treatments				
Creatinine (mg/dL)	0.5-1.5	0.5	0.5	0.5	0.5	0.03
BUN (mg/dL)[2]	6.0-30.0	11.3	10.5	12.1	11.6	0.85
Total protein (g/dL)	5.1-7.0	6.0	6.0	6.0	6.0	0.14
Albumin (g/dL)	2.5-3.8	3.3	3.3	3.3	3.3	0.06
Globulin (g/dL)	2.7-4.4	2.7	2.7	2.7	2.7	0.11
Ca (mg/dL)	7.6-11.4	10.4	10.5	10.4	10.4	0.11
P (mg/dL)	2.7-5.2	4.1	4.0	4.1	3.9	0.19
Na (mmol/L)	141-152	144.9	144.5	145.1	144.8	0.454
K (mmol/L)	3.9-5.5	4.5	4.5	4.4	4.5	0.08
Na:K ratio	28-36	32.7	32.4	33.1	32.6	0.61
Cl (mmol/L)	107-118	109.2	109.0	109.3	109.0	0.71
Glucose (mg/dL)	68-126	80.5	78.1	75.5	79.1	3.44
Total Bilirubin (mg/dL)	0.1-0.3	0.2	0.2	0.2	0.2	0.01
Cholesterol (mg/dL)	129-297	205.1	191.9	205.3	197.0	17.93
Triglycerides (mg/dL)	32-154	81.4	69.6	73.4	66.4	12.76
Bicarbonate (mmol/L)	16-24	22.3	22.6	22.5	22.8	0.55

[1]University of Illinois Veterinary Diagnostic Laboratory Reference Ranges.
[2]BUN: blood urea nitrogen.

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CHAPTER 4

EXTRUDED FELINE DIETS FORMULATED WITH HIGH INCLUSION OF SOYBEAN HULLS: EFFECTS ON APPARENT TOTAL TRACT MACRONUTRIENT DIGESTIBILITY, FECAL QUALITY, AND FECAL FERMENTATIVE END-PRODUCT CONCENTRATIONS

ABSTRACT

Dietary fibers have gained renewed interest in companion animal nutrition as a means to manage pet obesity and improve gut and host health. Soybean hulls (**SBH**), a co-product of the soybean oil extraction process, is an accessible and economical fiber source. However, limited research is available on the use of SBH in feline nutrition. Thus, the aim of this study was to determine the effects of a high SBH inclusion level on daily food intake, apparent total tract (**ATT**) macronutrient digestibility, fecal quality, and fecal fermentative end-products in diets of adult cats. Four diets were formulated with either SBH, beet pulp (**BP**), or cellulose (**CL**) as the main source of dietary fiber, with the contol diet formulated with no added fiber (**NF**). The fiber treatments were formulated to achieve approximately 15% total dietary fiber (**TDF**). Eight adult male cats (mean age = 10.5 yr ± 0.1; mean BW = 6.1 ± 0.8 kg) were used in a replicated 4x4 Latin square design. Each period consisted of 14 d, with 10 d of diet adaptation followed by 4 d of total fecal and urine collections. Food was offered twice daily and cats were fed to maintain body weight. Food intake on a dry matter basis (**DMB**) was lower ($P < 0.05$) in cats fed BP (55.2 g/d) when compared to SBH (70.8 g/d). As-is fecal output did not differ in cats fed BP or SBH and, when expressed on a DMB, fecal output did not differ among fiber treatments. The ATT digestibility of DM, OM, and GE were greater ($P < 0.05$) in cats fed NF when compared to those fed BP, CL, or SBH. Cats fed CL had the greatest ($P < 0.05$) ATT CP digestibility (88.5%), followed by cats fed NF(84.9) and SBH (81.7%) with the lowest values (77%) noted for cats fed

BP. Acid hydrolyzed fat (**AHF**) digestibility was greater for cats fed CL (92.9%) than for cats fed BP (86.9%) and SBH (88.6%). The TDF ATT digestibility was lowest for cats fed NF and CL (8.5 and 15.1%, respectively), followed by SBH (18.0%), with BP having the highest digestibility (33.7%). Total short-chain fatty acid concentration was greatest in cats fed BP (699.7 μmole/g) when compared to the other three treatments, while phenol and indole concentrations did not differ among treatments. In conclusion, a high inclusion level (15% TDF) of SBH appears possible in diets for adult cats, resulting in no negative effects on food acceptability with similar ATT macronutrient digestibility and fecal characteristics when compared to BP and CL.

INTRODUCTION

Dietary fiber is not nutritionally required by the adult cat (NRC, 2006). As an obligate carnivore, the nutritional relevance of dietary fiber for the domestic cat has been overlooked until recently. However, dietary fibers can provide a multitude of health benefits such as elicitingsatiety and aiding in weight loss, blunting the postprandial glycemic response, improving gut health and fecal quality, and increasing fecal short-chain fatty acid concentrations (Banta et al., 1979; Fahey et al., 1992; Massimino et al., 1998; Swanson et al., 2002; German, 2006; den Besten et al., 2013).

Soybean hulls (**SBH**) are a fiber-rich co-product of the soybean oil extraction process. In the U.S., soybean production is increasing, and it is followed by a steady decrease in yearly costs (USDA, 2017). According to the USDA's National Agricultural Statistics Services, soybean production in 2017 was forecast at approximately 4.4 billion bushels, from which it can be estimated about 220 million bushels of SBH (NASS/USDA, 2017). Historically, SBH have been partially reintroduced into soybean meal. However, increasing demand for soybean meal with greater protein content has resulted in SBH to be readily available for ruminant and monogastric nutrition (Extension, 2008). Previous research evaluating SBH has been done in canines, however there is a lack of research evaluating SBH in feline diets. Therefore, the objective of this study was to evaluate the effects of SBH on fecal fermentative end-products and ATT macronutrient digestibility of cats compared to a no fiber diet (control) and two added dietary fiber diets, beet pulp and cellulose. It was hypothesized that cats fed a SBH diet would exhibit intermediate macronutrient ATT digestibility and fecal fermentative end-product concentrations when contrasted with beet pulp and cellulose.

MATERIALS AND METHODS

Animals and Diets

This study used eight adult neutered male domestic shorthair cats (mean age = 10.5 ± 0.1 yr; mean body weight = 6.1 ± 0.8 kg). The cats were housed in a temperature-controlled room at the Edward R. Madigan Laboratory at the University of Illinois at Urbana-Champaign, under a 14 h light: 10 h dark schedule. All cats were group housed with the exception of 2 h, twice daily, for feeding (0800-1000 and 1500-1700). All cats were given free access to water at all times. All animal care procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to animal experimentation.

Four experimental diets were formulated to contain no additional fiber (**NF**), beet pulp (**BP**), cellulose (**CL**), or SBH as the main sources of dietary fiber (**Table 4.1**). Each source of dietary fiber was added at the expense of chicken by-product meal and brewer’s rice to achieve isonitrogenous and isocaloric diets (**Table 4.2**) and targeting approximately 30% crude protein

(**CP**), 15% acid hydrolyzed fat (**AHF**), and 15% total dietary fiber (**TDF**), except for the NF diet that was formulated to have 5% TDF. The diets were formulated to be complete and balanced according to AAFCO (2016) for adult cats at maintenance. The diets were extruded at the Kansas State University Bioprocessing and Industrial Value-Added Program facility (Manhattan, KS). Food intake was individualized based on metabolic body size and cats were fed to maintain body weight based on previous food intake records.

Experimental Design

A replicated 4x4 balanced Latin square design was used. Each experimental period consisted of 14 d; 10 d of diet adaptation, followed by 4 d of total fecal and urine collection. During the experimental period, cats were fed twice daily (0800 and 1500) and allowed 2 h to eat. Food intake and refusals were recorded after each meal. During the collection phase, all feces were collected, scored (1=dry, hard feces to 5= diarrhea; a score of 2-3 considered ideal), and stored in a -20°C freezer until analysis to determine apparent total tract (**ATT**) macronutrient digestibility. Additionally, during the collection period, one fresh fecal sample was collected from each cat within 15 min of defecation. The fresh sample was scored, weighed, and analyzed for pH. The sample then was aliquoted out to be measured for fermentative end-product concentrations, including short-chain fatty acids (**SCFA**), branched-chain fatty acids (**BCFA**), phenols, indoles, and ammonia. One aliquot was placed in 2 N hydrochloric acid and frozen at -20°C until analysis of SCFA, BCFA, and ammonia concentrations. Two aliquots were collected for measurement of phenols and indoles and frozen at -20°C until analysis. Finally, two aliquots were collected to determine dry matter. Total urine output was collected from d 11-14 and the volume and weight recorded. Urine samples were collected in containers containing 5 mL 2 N hydrochloric acid for immediate acidification upon urination. Acidified urine samples were subsampled (25% of each sample retained) and stored at -20°C until analysis. Additionally, at the end of each period, one fasted blood sample (6 mL) from each cat was collected to evaluate serum chemistry profiles and complete blood count. Blood was collected in BD Vacutainer serum separator tubes and EDTA tubes (Becton, Dickinson and Company, Franklin Lakes, NJ), respectively. Blood and serum samples were analyzed by the University of Illinois Veterinary School Diagnostics Laboratory using a Hitachi 911 clinical chemistry analyzer (Roche Diagnostics, Indianapolis, IN).

Sample Preparation and Chemical Analyses

Food and fecal samples were dried at 55°C in a forced-air oven. Diet and fecal samples were then ground in a Wiley mill (model 4, Thomas Scientific, Swedesboro, NJ) through a 2-mm screen and then analyzed for dry matter (**DM**), organic matter (**OM**), and ash according to AOAC (2006; methods 934.01 and 942.05). Crude protein content of diets and feces was calculated from Leco (TruMac N, Leco Corporation, St. Joseph, MI) total nitrogen values according to AOAC (2006; method 992.15). Total lipid content (**AHF**) was determined according to the methods of the American Association of Cereal Chemists (1983) and Budde (1952). Diet, feces, and urine were analyzed for gross energy (**GE**), measured by bomb calorimetry (Model 6200, Parr Instruments Co., Moline, IL). Urine GE values were used to calculate metabolizable energy (**ME**). Fecal and diet TDF were measured according to Prosky et al. (1992).

Fecal ammonia concentrations were measured according to the method of Chaney and Marbach (1962). Fecal phenol and indole concentrations were determined using gas chromatography according to the method by Flickinger et al. (2003). Fecal SCFA and BCFA concentrations were determined using gas chromatography according to Erwin et al. (1961).

Statistical Analyses

All data were analyzed using the Mixed Models procedure of SAS. Fecal score was analyzed using the GLIMMIX procedure of SAS. PROC UNIVARIATE was used to analyze for data normality (SAS Institute Inc., version® 9.4, Cary, NC). The model contained the fixed effect of diet and the random effect of cat. Differences among treatments were determined using a Fisher-protected least significant difference test with a Tukey adjustment to control for type-1 experiment-wise error. Reported pooled standard errors of the mean (**SEM**) were determined according to the Mixed Models procedure of SAS. A probability of $P \leq 0.05$ was accepted as statistically significant.

RESULTS AND DISCUSSION

Diet, Food Intake, and Fecal Characteristics

All four experimental diets were similar in chemical composition (**Table 4.2**). Dry matter content ranged from 92.2 to 95.4%. On a DM basis, all diets contained approximately 92% OM and an average of 30.9% CP. Fat content varied slightly among treatments with CL having the highest AHF content (16.4%), SBH and BP having intermediate AHF content (15.2 and 14.5%, respectively), and NF having the lowest AHF content (13.7%). These values are reflected in the GE content of the diets. The variation observed in the AHF content of these diets was due to deviations in the amount of fat dispersed on the kibbles during the fat coating step that could not be further controlled in the pilot plant. Additionally, due to low diet acceptance by the cats prior to the start of the study, the diets were coated with additional white choice grease and palatant (AFB BioFlavor F25003, St. Charles, MO) at 4% and 2% inclusion rates, respectively. The additional coating did not completely adhere to all of the diets, resulting in the slightly varying concentrations of AHF content. However, this should have negligible effect on the findings of this research, as there was an AHF difference of less than 3 percentage units among the dietary treatments. The three fiber treatments were formulated to have approximately 15% TDF and the NF treatment to have 5% TDF. The SBH and BP treatments had slightly higher TDF contents (16.6 and 17.1%, respectively), but this is not surprising as TDF content of these dietary fibers can vary depending on the source (Fahey et al., 1990; Sunvold et al., 1995a; Cole et al., 1999). Also, as expected, the insoluble and soluble fractions differed among fiber treatments. The BP diet contained the highest amount of soluble fiber (5.9%) compared to the CL (1.5%) and SBH (1.0%) diets.

Food intake (g/d, DM basis) differed ($P < 0.05$) among treatments (**Table 4.3**). Cats fed SBH had higher ($P < 0.05$) food intake (70.8 g/d) compared to those fed BP (55.2 g/d). This was due to greater food refusals by the cats fed BP. Decrease in food palatability was expected with greater TDF content of the diets. However, based on our findings, it appears that cats did not tolerate the high levels of TDF only in the BP diet compared to other treatments. Previous research evaluating up to 12.5% BP and 10% CL in diets of adult cats has not observed negative effects on food intake (Sunvold et al., 1995b). Despite the lower food intake by cats fed the BP diet, no significant differences in BW was observed among dietary treatments. This was likely due to the short experimental period (14 d). However, this finding must be considered when developing commercial pet foods at this high level of TDF content, as they can lead to suboptimal intake of essential nutrients and potential nutrient deficiencies over time.

Fecal score did not differ among fiber treatments (mean = 2.2) and remained within the acceptable fecal score range using a 5-point scale. Cats fed the NF treatment had a higher ($P < 0.05$) fecal score (2.8) than those fed the fiber treatments. Similar fecal scores were previously observed in cats (2.3) fed a diet containing 12.5% BP corresponding to 10.6% TDF (Sunvold et al., 1995b). However, in that same study, a lower fecal score (1.8) was reported for cats fed a diet with 8.1% CL and 11.2% TDF. As-is fecal output (g/d) was greater ($P < 0.05$) for cats fed BP (50.5 g/d) in comparison to cats fed CL (33.5 g/d), and NF (28.1 g/d), whereas SBH (45.6 g/d) did not differ from the other fiber treatments. When daily fecal output was converter to DM basis, fecal output (g/d) did not differ among fiber treatments. This indicates that BP had a greater water holding capacity due to its higher concentration of soluble fibers. Previous research in canines and sows fed diets containing BP have also reported this water holding capacity (Burkhalter et al., 2001; Serena et al., 2008).

Apparent Total Tract Macronutrient and Energy Digestibilities

Apparent total tract digestibilities by cats fed the experimental diets are listed in **Table 4.4**. Cats fed the BP, CL, and SBH treatments had lower ($P < 0.05$) ATT digestibilities of DM (74.5, 78.4, and 75.4%, respectively) and OM (77.9, 81.1, and 78.5%, respectively) when compared to the NF diet (DM: 85.5 and OM: 88.8%). Lower DM (70.9%) and OM (74.7%) digestibility values were reported by Fischer et al. (2012) when overweight cats were fed a diet containing 15.5% BP and 25.6% TDF. In contrast, Sunvold et al. (1995b) reported greater coefficients of DM and OM digestibility in cats fed diets containing 12.5% BP (DM: 80.4% and OM: 83.8%) and 8.1% CL (DM: 81.0% and OM: 83.5%). Dietary fiber has been reported to reduce OM digestibility in cats (Kienzle et al., 1991). In the current study, TDF content of the fiber-supplemented diets was high compared with previously reported data.

Cats fed the BP diet had lower ($P < 0.05$) ATT CP digestibility (77.2%) among experimental treatments. Cats fed the NF treatment did not differ in CP digestibility (84.9%) when compared to cats fed CL or SBH, however, CL had greater ($P < 0.05$) CP digestibility than

SBH (88.5 and 81.7%, respectively). Even though cats fed BP had lower CP digestibility, BP is higher in soluble, fermentable fibers compared to other fiber treatments. This fermentable property of BP fiber might have caused an increase microbial N due to fermentative process in the hindgut, which were then partially excreted in the feces thus causing a false perception of undigested protein and decreased ATT CP digestibility (Sunvold et al., 1995a; Rossoni Serão and Fahey, 2013). Similar rationale can be applied for the reduced CP digestibility observed in cats fed the SBH diet.

Cats fed NF, BP, and SBH treatments had no differences ($P > 0.05$) in ATT AHF digestibility (89.9, 86.9, and 88.6%, respectively). Cats fed CL had the highest AHF digestibility (92.9%) that could be due to the higher AHF concentration in the CL diet. Previous studies have reported slightly higher AHF digestibility in adult cats. Barry et al. (2010) observed higher AHF digestibility (95.8%) in a diet containing 4.0% CL and 7.9% TDF. Additionally, Sunvold et al. (1995b) fed NF, BP, and CL diets to cats that contained 1.7, 10.6, and 11.2% TDF and higher AHF digestibility was observed (93.9, 91.5, and 95.0%, respectively). However, the previous research examined diets containing lower TDF values. As TDF increases, ATT nutrient digestibility tends to decrease. Furthermore, the current study used choice white grease versus more common fat sources, such as chicken fat or vegetable oil. Choice white grease contains greater concentrations of saturated fatty acids compared to other fat sources. For comparison, choice white grease has an unsaturated:saturated ratio of 0.31, compared to beef tallow (0.08), poultry fat (0.71) and soybean oil (4.07; NRC, 2006). Diets containing higher amounts of unsaturated fats (i.e., soybean oil) have been demonstrated to have higher fat digestibility by dogs (Marx et al., 2015), which may have influenced the coefficients of AHF digestibility observed in the current study.

Cats fed BP had the greatest (*P < 0.05*) ATT TDF digestibility (33.7%), followed by SBH (18.0%), and CL (15.1%). Cats fed the NF treatments had the lowest TDF digestibility (8.5%). These are expected results as CL and SBH have a higher ratio of insoluble to soluble fiber (13.6:1.5 and 15.6:1.0) in contrast with BP (11.2:5.9). Similar BP digestibility coefficients were reported when cats were fed a diet containing 15.5% BP (Fischer et al., 2012). Sunvold et al. (1995b) reported TDF digestibility values that were approximately half of what we found (8.9%) to cats fed a diet containing 8.1% CL. However, they reported similar TDF digestibility in cats fed diets containing BP and NF, 38.2 and 5.3%, respectively. Insoluble fibers are less fermentable in the large intestine of monogastric animals, especially in cats where the large intestine is very short. This resulted in most of the insoluble fiber not being fermented by the microbiota and excreted in the feces.

Similar to DM and OM digestibilities, digestible energy (**DE**) was similar for cats fed the three fiber treatments and lower ($P < 0.05$) than cats fed the NF treatment. Slightly lower DE (75.7%) was previously reported in cats fed a BP diet containing 15.5% BP (Fischer et al., 2012). The ME values were the lowest ($P < 0.05$) for cats fed BP, followed by SBH and CL, with NF having the highest ME. These results are expected as higher fiber diets have been observed to interfere with digestibility of energy (Earle et al., 1998; Fahey Jr. et al., 2004). Moreover, the lower DE and ME values are not a negative attribute to the fibrous diets. Fischer et al. (2012) and Weber et al. (2007) suggest that higher TDF levels could lead to improved satiety and have weight loss and management applications due to its lower DE and ME digestibilities.

Fecal Fermentative End-Products and Serum Chemistry

It is assumed that the higher amounts of fermentative end-products measured in the feces is a reflection of the colonic fermentative process in the large intestine in the cat. There were no differences among treatments in regards to fecal ammonia (mean = 119.2 μmole/g, DMB) or total phenol and indole concentrations (mean = 1.8 μmole/g, DMB; **Table 4.5**). This contrasts with previous research that observed higher 4-methylphenol and indole concentrations in cats fed a CL diet that contained 7.9% TDF (Fischer et al., 2012). Additionally, cats fed BP, SBH, and NF diets had a similar fecal pH, ranging from 5.5 to 5.7, whereas a greater fecal pH (6.0) was observed in cats fed the CL diet. Lower fecal pH (5.9) has been reported to reduce the resorption of ammonia in dogs, causing a faulty greater fecal ammonia concentration (Matsuoka et al., 1990). This is in agreement with our data as BP, SBH, and NF diets had an observed lower pH and numerically higher fecal ammonia concentrations when compared to cats fed CL.

Cats fed BP had greater ($P < 0.05$) total fecal SCFA concentration (699.7 μmole/g, DMB) than the other three treatments, suggesting an increase in saccharolytic fermentation. Cats fed NF and SBH had intermediate total SCFA concentrations (456.0 and 422.7 μmole/g DMB, respectively), followed by CL-fed cats (231.6 μmole/g, DMB). Similar total fecal SCFA concentrations were observed in cats fed CL (Barry et al., 2010) and in cats fed BP (Fischer et al., 2012). When total SCFA concentrations were divided into individual SCFA (i.e., acetate, propionate, and butyrate), cats fed BP had the greatest ($P < 0.05$) acetate and propionate concentration compared to the other three experimental diets, which was also observed by Fischer et al. (2012). In *in vitro* studies completed using cat fecal inoculum, BP was also observed to have higher acetate, propionate, and total SCFA production (Sunvold et al., 1995a,b). Cats fed the NF diet had the greatest ($P < 0.05$) amount of butyrate concentration (155.2 μmole/g, DMB), followed by BP (101.5 μmole/g, DMB) and SBH (72.1 μmole/g, DMB), while cats fed CL had the lowest butyrate concentration (38.0 μmole/g, DMB). Although cats fed SBH appear to be producing less butyrate numerically when compared to BP, proportionally it is producing more (17.1%) when compared to BP (14.5%) and CL (16.4%). Butyrate is a key energy source for colonocytes and preferentially taken up and used compared to acetate and propionate (Slavin, 2013). Additionally, butyrate has been observed in humans to have potential protective qualities against diseases (Christl et al., 1996).

Cats fed CL had lower ($P < 0.05$) fecal BCFA concentrations (11.9 μmole/g, DMB) in contrast with cats fed the NF, BP, or SBH treatments (avg. = 25.3 μmole/g, DMB). Low BCFA concentrations indicate that there was less protein fermentation, therefore more dietary protein was being digested by the animal. This data contrasts with previous research, as Fischer et al. (2012) reported BCFA concentrations in cats fed CL to reach 63.3 μmole/g, DM basis. Phenols, indoles, ammonia, and BCFA are putrefactive compounds that result from bacterial fermentation in the hindgut and cause undesirable fecal characteristics, including foul-smelling feces. This can be an unappealing quality from a pet owner's standpoint (Miner and Hazen, 1969; O'Neill and Phillips, 1992). Branched-chain fatty acids are produced when carbohydrates are limiting to the large intestinal microbes, resulting in branched-chain amino acids (**AA**) being fermented (Macfarlane et al., 1992). Previous research has reported that increasing the amount of rapidly fermentable fibers (i.e. short-chain fructooligosaccharides, galactooligosaccharides, and pectin) results in an increase in peptides and AA produced in the proximal colon, followed by microbes fermenting the products to generate the putrefactive compounds (Barry et al., 2010; Kanakupt et al., 2011). In the current study, there were no differences between BP, the fiber gold standard, and SBH, illustrating the benefit of SBH as a valuable dietary fiber source in pet food.

The serum chemistry profiles were analyzed for this study as a health check to ensure that the experimental diets were not detrimental to cat health (**Table 4.6)**. There were no differences ($P > 0.05$) among treatments and all values, aside from creatinine, were within the corresponding reference ranges provided by the University of Illinois Veterinary Diagnostics Laboratory. The creatinine values above the reference range observed for BP, CL, and SBH treatments are reflective of the age of the cats (Ross et al., 2006). There was no effect of treatment on serum creatinine concentration. Due to the age of the cats (~ 10 yr) used in this study, a gradual increase in serum creatinine is expected. These cats were clinically healthy, and with no signs of renal dysfunction or failure. Complete blood count results also were within reference ranges for all cats (data not shown).

IMPLICATIONS

Results of this study indicated that cats fed SBH were similar when compared to the other fiber treatments, BP and CL. Cats fed SBH had similar ATT macronutrient digestibilities with no detrimental effects on fecal quality or health status. The SBH diet was the most well-accepted diet in terms of food intake. Additionally, SBH produced similar proportions of SCFA when compared to BP and did not differ in putrefactive compounds when compared to NF and BP treatments. Although the SBH diet had a higher insoluble:soluble fiber ratio than CL, fecal fermentative end-product results indicated that the SBH diet had similar fermentation patterns to the BP diet, suggesting that the insoluble fraction of SBH might be more fermentable than CL. Based on the findings of this research, SBH appear to be a suitable dietary fiber source in feline diets even when fed at high concentrations. As such, SBH can be used as an economical, readily available, and sustainable dietary fiber source in adult cat food formulations. It also can have further applications in weight maintenance diets for overweight cats, as the addition of palatable fibers can reduce dietary energy content while maintaining normal food intake (Fekete et al., 2001).

TABLES

Table 4.1. Ingredient composition of experimental diets containing select fiber sources.

	Treatments			
Item, % DM basis	**No Fiber**	**Beet Pulp**	**Cellulose**	**Soybean Hulls**
Chicken by-product meal	29.1	29.6	31.1	28.7
Corn gluten meal	7.7	7.7	7.7	7.7
Brewer's rice	43.0	26.9	31.3	29.4
Corn, whole, ground	3.7	3.7	3.7	3.7
Choice white grease	8.0	8.0	8.0	8.0
Coating – palatant[1]	2.0	2.0	2.0	2.0
Coating – choice white grease	4.0	4.0	4.0	4.0
Beet pulp[2]	-	15.5	-	-
Cellulose[2]	-	-	9.6	-
Soybean hulls[2]	-	-	-	14.0
Salt	0.5	0.5	0.5	0.5
Potassium chloride	0.45	0.45	0.45	0.45
Taurine	0.2	0.2	0.2	0.2
Mineral premix[3]	0.18	0.18	0.18	0.18
Vitamin premix[4]	0.18	0.18	0.18	0.18

[1]AFB BioFlavor F25003, St. Charles, MO.
[2]Lortscher Animal Nutrition Inc., Bern, KS.
[3]Provided per kg diet: 17.4 mg manganese ($MnSO_4$), 284.3 mg iron ($FeSO_4$), 17.2 mg copper ($CuSO_4$), 2.2 mg cobalt ($CoSO_4$), 166.3 mg zinc ($ZnSO_4$), 7.5 mg iodine (KI), and 0.2 mg selenium (Na_2SeO_3).
[4]Provided per kg diet: 11,000 IU vitamin A Acetate; 900 IU vitamin D3; 57.5 IU vitamin E Acetate; 0.6 mg vitamin K; 7.6 mg thiamine HCl; 11.9 mg riboflavin; 18.5 mg pantothenic acid, d, calcium; 93.2 mg niacin; 6.6 mg pyridoxine HCl; 12.4 mg biotin; 1,142.1 µg folic acid; 164.9 µg vitamin B12, 0.1% mannitol.

Table 4.2. Analyzed chemical composition and energy content of experimental diets containing select fiber sources.

	Treatments			
Item	**No Fiber**	**Beet Pulp**	**Cellulose**	**Soybean Hulls**
Dry matter (%)	92.2	93.9	95.4	94.8
	---------- %, DM basis ----------			
Organic matter (%)	92.4	92.4	92.3	92.1
Crude protein (%)	30.8	30.2	31.9	30.7
Acid-hydrolyzed fat (%)	13.7	14.5	16.4	15.2
TDF[1] (%)	4.5	17.1	15.1	16.6
Insoluble (%)	2.7	11.2	13.6	15.6
Soluble (%)	1.8	5.9	1.5	1.0
GE[1] (kcal/g; measured)	5.2	5.2	5.3	5.2
ME[2] (kcal/g; calculated)	3.8	3.4	3.5	3.4

[1]TDF = total dietary fiber; GE = gross energy (measured by bomb calorimetry).
[2]ME= metabolizable energy; ME = 8.5 kcal ME/g fat + 3.5 kcal ME/g CP + 3.5 kcal ME/g nitrogen-free extract.

Table 4.3. Food intake and fecal characteristics of cats fed diets containing selected fiber sources.

Item	Treatments				SEM	*P*-value
	No Fiber	Beet Pulp	Cellulose	Soybean Hulls		
Food intake						
g/d, DM basis	69.7[ab]	55.2[b]	70.6[ab]	70.8[a]	5.43	0.0320
Fecal characteristics/output						
Fecal score[2]	2.8[a]	2.3[b]	2.0[b]	2.2[b]	0.09	0.0001
Fecal output, as-is (g/d)	28.1[c]	50.5[a]	33.5[bc]	45.6[ab]	4.15	0.0024
Fecal output, DMB (g/d)	9.8[b]	12.8[ab]	16.0[ab]	17.9[a]	1.73	0.0118
Fecal DM (%)	35.0[bc]	30.6[c]	47.8[a]	38.9[b]	1.30	0.0001

[1]Fecal scores: 1= hard, dry pellets; small hard mass; 2 = hard formed, remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured.
[a-c] Means in the same row without common superscript letters denote a significant difference ($P < 0.05$).

Table 4.4. Total tract apparent macronutrient and energy digestibilities of cats fed diets containing selected fiber sources.

	Treatments					
Item	**No Fiber**	**Beet Pulp**	**Cellulose**	**Soybean Hulls**	**SEM**	***P*-value**
Nutrient and energy digestibilities, %						
Dry matter	85.5[a]	74.5[b]	78.4[b]	75.4[b]	1.55	0.0001
			---------- %, DM basis ----------			
Organic matter	88.8[a]	77.9[b]	81.1[b]	78.5[b]	1.37	0.0001
Crude protein	84.9[ab]	77.2[c]	88.5[a]	81.7[b]	1.13	0.0001
Acid hydrolyzed fat	89.9[ab]	86.9[b]	92.9[a]	88.6[b]	1.63	0.0001
TDF[1]	8.5[b]	33.7[a]	15.1[b]	18.0[ab]	4.88	0.0113
DE[1]	88.6[a]	78.6[b]	83.7[b]	79.9[b]	1.31	0.0001
ME[1]	82.3[a]	70.9[c]	78.2[ab]	73.6[bc]	1.52	0.0001

[1]TDF = total dietary fiber; DE = digestible energy; ME = metabolizable energy.

[a-c] Means in the same row without common superscript letters denote a significant difference ($P < 0.05$).

Table 4.5. Fecal fermentative end-products of cats fed diets containing select fiber sources.

	Treatments					
Item (µmole/g DM basis)	**No Fiber**	**Beet Pulp**	**Cellulose**	**Soybean Hulls**	**SEM**	***P*-value**
Fecal pH	5.5^{b}	5.7^{ab}	6.0^{a}	5.7^{ab}	0.11	0.0059
Ammonia	125.5	126.0	94.9	130.5	17.24	0.2608
Phenols & Indoles						
Total Phenols/Indoles	1.7	2.6	1.1	1.9	0.89	0.6773
4-Methylphenol	0.5	1.2	0.4	0.5	0.52	0.9714
Indole	1.2	1.4	0.7	1.4	0.42	0.3638
SCFA[1]						
Total SCFA	456.0^{b}	699.7^{a}	231.6^{c}	422.7^{bc}	58.41	0.0001
Acetate	238.1^{b}	459.2^{a}	146.4^{b}	274.3^{b}	42.09	0.0001
Propionate	62.7^{b}	139.0^{a}	47.2^{b}	76.2^{b}	13.22	0.0002
Butyrate	155.2^{a}	101.5^{b}	38.0^{c}	72.1^{bc}	16.70	0.0001
BCFA[1]						
Total BCFA	24.6^{a}	24.6^{a}	11.9^{b}	26.8^{a}	4.81	0.0029
Isobutyrate	6.6^{a}	6.0^{ab}	3.0^{b}	7.6^{a}	1.48	0.0075
Isovalerate	11.5^{a}	10.1^{ab}	5.0^{b}	13.4^{a}	2.65	0.0017
Valerate	6.6	8.5	4.0	5.8	2.72	0.3202

[1]SCFA = short chain fatty acids; BCFA = branched chain fatty acids.

[a-c] Means in the same row without common superscript letters denote a significant difference ($P < 0.05$).

Table 4.6. Serum metabolites of cats fed diets containing select fiber sources.

Item	Reference Range[1]	Treatments				SEM
		No Fiber	Beet Pulp	Cellulose	Soybean Hulls	
Creatinine (mg/dL)	0.4-1.6	1.6	1.8	1.7	1.7	0.09
BUN (mg/dL)[2]	18-38	23.3	21.4	22.3	23.5	1.03
Total protein (g/dL)	5.8-8.0	6.6	6.5	6.5	6.7	0.20
Albumin (g/dL)	2.8-4.1	3.1	3.1	3.1	3.2	0.08
Globulin (g/dL)	2.6-5.1	3.5	3.3	3.4	3.5	0.14
Ca (mg/dL)	8.8-10.2	9.3	9.2	9.2	9.3	0.14
P (mg/dL)	3.2-5.3	4.1	3.8	4.1	3.8	0.17
Na (mmol/L)	145-157	148.6	149.0	149.7	149.3	0.45
K (mmol/L)	3.6-5.3	4.7	4.4	4.5	4.5	0.10
Na:K ratio	28-36	32.0	33.9	33.3	33.1	0.77
Cl (mmol/L)	109-126	117.0	116.4	118.0	116.8	0.67
Glucose (mg/dL)	60-122	83.3	77.1	83.8	78.9	3.69
Total Bilirubin (mg/dL)	0.0-0.3	0.1	0.1	0.1	0.1	0.02
Cholesterol (mg/dL)	66-160	147.5	140.5	158.4	143.8	14.32
Triglycerides (mg/dL)	21-166	33.5	34.0	41.3	35.1	3.82
Bicarbonate (mmol/L)	12.0-21.0	17.5	18.3	16.7	16.9	0.56

[1]University of Illinois Veterinary Diagnostic Laboratory Reference Ranges.
[2]BUN: blood urea nitrogen.

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CHAPTER 5

SUMMARY

There has been limited research evaluating the effects of soybean hulls (SBH) in canine and feline diets. The current studies aimed to evaluate the effects of high inclusion of dietary SBH on fecal quality, food intake, macronutrient apparent total tract (ATT) digestibility, and fecal fermentative end-products of adult dogs and cats. Experimental diets were formulated to have a greater TDF content (~ 15%) in comparison to standard premium commercial pet foods (≤ 8%).

There was no observed negative effect on food intake for both the cats and the dogs. Cats are normally not very tolerant of high fiber diets, and while there were some refusals, the SBH was generally well accepted by the cats, resulting in adequate food intake to fulfill their nutritional requirements. In addition to positive food acceptance, fecal output and score were not affected by inclusion of SBH. For dogs and cats, fecal scores were within the ideal fecal score range (2.5-3.0) on a 5-point scale (1= hard, dry pellets; small hard mass; 2 = hard formed, remains firm and soft; 3 = soft, formed and moist stool, retains shape; 4 = soft, unformed stool; assumes shape of container; 5 = watery, liquid that can be poured).

Apparent total-tract macronutrient digestibility (ATTD) was not negatively affected with the addition of SBH in the diets of the dogs and cats. When comparing ATT macronutrient digestibility between dogs and cats fed SBH, digestibility values were very similar. In addition, similar physiological results were observed in animals fed SBH in comparison with animals fed BP, which is considered the gold standard fiber source by the pet food industry (Godoy et al., 2013).

There were observed differences in fecal fermentative end-product concentrations in both dogs and cats fed SBH, with larger shifts observed in the dogs in comparison to cats. Soy often is associated with flatulence (Grieshop and Fahey, 2000). However, dogs fed SBH had lower putrefactive compound concentrations (i.e. total phenols and indoles and branched-chain fatty acids [BCFA]) in the feces when compared to dogs fed cellulose (CL) and the no fiber (NF) control diet. In cats, there were no differences in total fecal phenol and indole concentrations among treatments, and SBH resulted in similar fecal branched-chain fatty acid concentrations to NF and BP. Cats fed SBH also had intermediate concentrations of short-chain fatty acids (SCFA) compared to the other fiber treatments. However, in dogs, total fecal SCFA concentrations did not differ from dogs fed BP and a beneficial increase was observed for acetate, propionate, and butyrate.

Based on these data, SBH is a viable dietary fiber source in canine and feline diets. Animals fed SBH performed similar to animals fed BP, especially the dogs. There could be various applications of SBH in the pet food industry as well. Soybean production in the United States has steadily increased followed by a steady decrease in production cost (USDA, 2017), resulting in SBH being readily available for use in companion animal diets. Diets formulated with SBH can be used for cost-effective diets, weight loss or management diets, or therapeutic diets where fiber is important factor in mitigating symptoms. Thus, SBH should be further investigated for its functional properties in canine and feline nutrition.

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