Evaluation of whole soybean as an ingredient in extruded dog diets

by

Hee Seong Kim

B.S., Sookmyung Women's University, 2015
M.S., Seoul National University, 2017

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Grain Science and Industry
College of Agriculture

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Abstract

Soybean use in pet food is low due to undeserved negative perceptions among some marketers and consumers. However, soybean is an excellent ingredient beyond protein for its fat and dietary fiber contribution to dogs. Utilizing internal fat in whole soybeans (WSB) may increase the energy density of dog diets while avoiding production issues, and the oligosaccharides (OS) may perform as prebiotics. The objective of this research was to determine the effects of increasing levels of WSB in dry dog foods. This work elaborated theoretical and practical aspects of extrusion processing, nutrient digestibility, *canine in vitro* microbial fermentation, palatability, and consumer sensory analysis. Whole soybeans were beneficial at providing elevated fat levels (versus soybean oil) into the extruder without causing critical issues in processing and product stability. The increased inclusion level of WSB did decrease the mechanical energy within the extruder from the intrinsic fat content. In addition, the mechanical energy input did not completely destroy the anti-nutritional factors at increasing WSB levels. The nutrient apparent total tract digestibility in dogs remained high (over 80%), but at higher inclusion of WSB, there was a slight linear decrease which might be attributed to the dietary fibers and residual anti-nutritional factors. The stool quality and palatability were not affected by WSB up to 30% in the formulas in dogs. The WSB diets increased hind-gut fermentation (increased short-chain fatty acids and decreased fecal pH) in dogs, which can be useful in high-fiber diets for geriatric, lower calorie diets for less-active, or gastrointestinal health. The canine *in vitro* microbial fermentation model further supported the WSB prebiotic effect, wherein greater butyrate production was noted for WSB than for beet pulp due to the fermentation of both oligosaccharides and soluble fiber components in WSB. For overall sensory analysis, WSB could replace proportional levels of brewers' rice, corn gluten meal, and chicken fat for consumers.

Subtle differences were noted in increased color and fracturability, and porosity, gritty, oily mouthcoating, and heated oil aftertaste decreased as the WSB inclusion level increased. Only a slight change in color liking was observed from the consumer study, with no effects on overall liking, appearance, size, shape, and aroma liking as the WSB levels increased. In addition, consumers still responded favorably to their dogs' liking scores for the WSB-containing diets. In conclusion, WSB inclusion of 10% in the diets appears to be the optimal level recommended when the diets were produced in the same condition as WSB 0% diet but were feasible in processing and feeding at levels up to 30%.

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differences were noted in increased color and fracturability, and porosity, gritty, oily mouthcoating, and heated oil aftertaste decreased as the WSB inclusion level increased. Only a slight change in color liking was observed from the consumer study, with no effects on overall liking, appearance, size, shape, and aroma liking as the WSB levels increased. In addition, consumers still responded favorably to their dogs' liking scores for the WSB-containing diets. In conclusion, WSB inclusion of 10% in the diets appears to be the optimal level recommended when the diets were produced in the same condition as WSB 0% diet but were feasible in processing and feeding at levels up to 30%.

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Dedication

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Chapter 1 - Evaluation of soy ingredients in pet foods applications: Systematic review

1.1 Abstract

Soybean use has been low in pet foods, even though they are an excellent source of protein, polyunsaturated fatty acids, and gut fermentable fibers. The purpose of this evaluation was to conduct a systematic review of the public literature to explore how soybeans have been researched for pet food applications since 2000 and to provide strengths, weaknesses, opportunities, and threats for soybeans in the pet food industry. A total of 44 articles were categorized by their research contents and narratively summarized in the results. When soybean-based products have been adequately processed to reduce the antinutritive factors, they are comparable to animal proteins in nutritional value, palatability, and functionality in pet food processing. We conclude that various food processing technologies and the versatility of soybean ingredients allow soy to have considerable inclusion potential in pet foods. More research with dietary soy ingredients regarding pet food processing, fermentation benefits on health, and consumer acceptance will be needed to understand soy's position in the future pet food industry.

1.2 Introduction

Soy ingredients including soy flour, soybean meal, soy protein concentrates, soybean oil, and other variations used in U.S. pet food totaled 534,069 tons in 2019, representing approximately 6.18% of the total 8,646,211 tons of pet food ingredients (IFEEDER, 2020). According to the summary quantities of total plant-related pet food ingredients, soybean meal was the third largest ingredient (427,155 tons) following corn (1,283,674 tons) and corn gluten meal (476,649 tons) (IFEEDER, 2020). Because cats are carnivores while dogs are omnivores,

the soybean meal and soybean oil volumes used in dog foods (344,751 and 2,414 tons) were higher than that in cat foods (82,404 and 479 tons), according to the report (IFEEDER, 2020). However, the soy flour volume used in dog foods (30,912 tons) was similar to that in cat foods (32,528 tons) (IFEEDER, 2020).

Soybeans are an excellent source of protein, polyunsaturated fatty acids (omega-6 and omega-3 fatty acids), and dietary fiber. The whole soybean contains approximately 38% crude protein (CP), 21% acid hydrolyzed ether extract (AHEE), and 20% total dietary fiber (TDF) on a dry matter basis (Kim and Aldrich, 2023). To increase the nutritional value of soy for different uses, various production processes are employed. According to National Research Council (2006), soybean hulls contain ~13% CP, soybean meal contains ~44% CP, and soybean meal without hulls contains ~48% CP. Additionally, soy protein concentrate (SPC) contains about 70% CP and soy protein isolate (SPI) contains about 90% CP. Either dehulled or whole soybeans go through an oil extraction process (solvent or mechanical), and the residues are processed in different ways to yield desirable soy ingredients.

Soybeans contain anti-nutritional factors including trypsin inhibitors, urease (Félix et al., 2020), and oligosaccharides (Stein et al., 2008), which may limit their use in pet food. However, most dry pet foods are produced using an extrusion process, and the heat processing deactivates most protease inhibitors (Riaz, 2000). Even though research and industry has demonstrated that soybeans have real value in pet diets, pet owners remain skeptical of soy's inclusion in pet foods due to its undeserved reputation as a poor-quality ingredient, according to many social media outlets. In other words, there is a gap in the translation of information to the consumers regarding the quality of soy ingredients in companion animals' diets.

Thus, this research asks the question: what effects, if any, do soy ingredients in dog or cat diets have on animal health and nutrition, palatability, feeding behavior, allergenicity, and extrusion processing? The most comprehensive review regarding soybean use in pet foods

dates back to Drackley (2000). Therefore, the objective of this systematic review was to explore the original research published since 2000 regarding soy in pet foods and to summarize the findings regarding its nutritional value. This review intends to determine whether there are issues that need to be addressed in regard to soy ingredients and their value to pet diets, or if the data are available and need to be better communicated to the public.

1.3 Materials and Methods

1.3.1 Study protocol

This systematic review of the public literature was undertaken to evaluate original research publications related to soy consumption by companion animals (dogs and cats) in the fields of nutrition, immunology and allergenicity, behavior, and pet food processing. The study criteria were developed by the authors, and research began in October 2022. Procedurally, this work was conducted similarly to the recent article by Vanelli et al. (2021).

1.3.2 Source and research information

The published research studies included in this review were found through searches in scientific databases. The searches were performed using the Web of Science, CAB Direct, and Scopus via a series of developed key words utilizing Boolean search terms. In the three databases selected, searches were conducted for documents that contained the terms (*soy**) AND (*dog* OR canine OR feline OR pet**) within the article title and (*process* OR antinutritional OR oligosaccharide* OR extru* OR nutri* OR sustainability OR digestibility OR allergen* OR trypsin* OR ferment* OR immuno* OR gmo OR vegetarian*) AND (*food* OR feed**) within all fields. The last data search was performed on October 4th, 2022.

1.3.3 Selection of studies and construction of databases

Only journal articles written in English and published since 2000 were selected. The following materials were excluded: book references, book chapters, and literature reviews. After duplicate removal, a total of 74 articles were selected for screening by reading titles and abstracts. Only the articles featuring dogs or cats that had a soybean component as a dietary treatment were selected.

After the qualified studies were selected, the articles were thoroughly read and reviewed to categorize them by their research topic. The categories were: animal health and nutrition, palatability, feeding behavior, allergenicity, and processing applications.

1.4 Literature review

1.4.1 Search and selection of studies

The results were organized into groups according which search and scientific database they resulted from, totaling 155 articles (Figure 1). The largest number of articles (66) were identified in the Scopus database, followed by CAB Direct (51), and Web of Science (38). The review of the articles began with the exclusion of non-English articles, which resulted in 143 papers to be evaluated. Twelve articles were excluded for language: 8 written in Portuguese, 3 written in Chinese, and 1 article written in Indonesian. Next, 69 duplicate articles were removed. Of the 74 remaining articles, 24 were excluded after the title and abstracts were read. Twenty-three of the discarded articles were not related to dogs or cats, but rather the search included them due to partial word matches (for example, ‘en“dog”enous’). Additionally, 1 article was not related to soybeans. Finally, the remaining 50 papers were read in full. Only 44 were consistent with the established selection criteria and were evaluated in this review. Six addressed ex-vivo blood work that did not have soy as a dietary component.

The remaining 44 articles were categorized by their research focus: animal health and nutrition (n=30), palatability (n=7), feeding behavior (n=2), immunology and allergenicity (n=10), and processing applications (n=4). Some articles (n=7) evaluated both nutrition and palatability of soy ingredients by pets, others (n=2) evaluated both nutrition and processing parameters of soy-including diets. There were 2 cat digestibility experiments studying mixed-breed cats (Carciofi et al., 2009) and shorthair cats (Detweiler et al., 2019), while Carciofi et al. (2009) contained studies on both dogs and cats. There were 40 dog studies with various breeds of dogs: purpose-bred dogs (Clapper et al., 2001), mongrel dogs (Yamka et el., 2003; Yamka et al., 2005a; Yamka et al., 2005b), mix-breed dogs (Yamka et al., 2006), Spitz dogs (Pawar and Pattanaik, 2009; Pattanaik and Kore, 2021), beagles (Carciofi et al., 2009; Félix et al., 2012; Félix et al., 2013a; Félix et al., 2013b; Tortorla et al., 2013; Beloshapka et al., 2016; Venturini et al., 2018; Félix et al., 2020), Labradors (Dhaliwal et al., 2016), hound dogs (Hill et al., 2011; Menniti et al., 2014), and a mix of breeds including Labrador, American Pit Bull Terrier, Weimaraner, Border Collie, Dachshund, and Great Dane (Marx et al., 2015). The remaining 3 studies examined only processing of pet food or the analysis of soy phytoestrogen concentration in dog foods, with no experimental animals needed.

1.4.2 Soy ingredients used in pet food

Various soy ingredients are used in pet foods depending on the processing and nutritional composition requirements (Table 1). The differences between the processing of soy ingredients is well described in a chapter by Rhee (2000) in “Soy in Animal Nutrition” and Félix et al. (2013a). Soybean meal (SBM) is produced by submitting dehulled ground soybeans to conditioning, flaking, oil extraction, desolventisation (hexane), and heating. Soy protein concentrate is obtained from SBM by treating it with an ethanol solution to remove soluble sugars and increase the concentration of the protein. In contrast, soybean protein isolate is made by separation of the protein from both soluble and insoluble carbohydrates using alkaline pH

adjustment, centrifugation, precipitation, drying, and membrane filtration (Van Krimpen et al., 2013). Hydrolyzed soy protein is obtained by enzymatically hydrolyzing defatted soy flakes using protease to increase protein solubility and hypoallergenicity. Micronizing is a process that cooks soybeans with the heat generated by vibrating molecules under infrared light.

Soybean products (reported as soy flour, raw soybeans, micronized whole soybeans, toasted soybeans) analyzed by the manuscripts included in this review had on average 920 ± 24.9 g/kg dry matter (DM), 51 ± 9.5 g/kg ash, 421 ± 66.6 g/kg CP, 194 ± 82.9 g/kg AHEE, 24 ± 0.2 MJ/kg gross energy (GE), and 42 ± 22.2 g/kg crude fiber (CF) on a DM basis. Soybean meal products (reported as soybean meal, low-oligosaccharide low-phytate soybean meal, regular soybean meal, high-protein soybean meal, defatted soybean meal) used in the reviewed pet foods studies had on average 908 ± 38.0 g/kg DM, 60 ± 12.5 g/kg ash, 462 ± 141.9 g/kg CP, 51 ± 35.3 g/kg AHEE, 20 ± 0.4 MJ/kg GE, and 42 ± 19.6 g/kg CF on a DM basis. Soybean protein concentrate products (reported as soy protein concentrate, hydrolyzed soy protein concentrate, soy protein isolate) used in pet foods had on average 933 ± 17.7 g/kg DM, 62 ± 13.0 g/kg ash, 705 ± 79.2 g/kg CP, 17 ± 11.7 g/kg AHEE, 21 ± 1.2 MJ/kg GE, and 37 ± 19.0 g/kg CF on a DM basis.

There were 7 manuscripts that reported the anti-nutritional factors and protein dispersibility index (PDI) of their experimental soy ingredients (Table 2). Among the anti-nutritional factors, urease activity was the most frequently studied in soy ingredients (n=14), followed by trypsin inhibitors (n=7), with only 2 data values for phytate concentrations. Levels of soy oligosaccharides such as stachyose and raffinose were reported in one manuscript (Félix et al., 2013b) with 5 data values for various soy ingredients. The PDI values for different soy ingredients were reported by Félix et al. (2012), Félix et al. (2013a), and Félix et al. (2013b). Each analytical value for each component varied by ingredient and by manuscript.

Soybean hulls have also been used as a soy-derived ingredient to provide fiber in pet food (Table 3). Soybean hulls (reported as hulls sourced from different companies) analyzed by the manuscripts included in this review had on average 917 ± 16.3 g/kg DM, 53 ± 3.0 g/kg ash, 130 ± 22.3 g/kg CP, 743 ± 58.5 g/kg TDF, 666 ± 44.9 g/kg IF, and 77 ± 28.9 g/kg SF on a DM basis. In addition, the ratio of insoluble fiber to soluble fiber in soybean hulls was on average 10 ± 3.4 on DM basis.

1.4.3 Impact of soy ingredients on animal health and nutrition

1.4.3.1 Soy ingredients and nutrient digestibility

The articles reporting research regarding animal health and nutrition were the most numerous (n=31). There were 20 manuscripts that measured apparent total tract digestibility (ATTD, %) of the diets in dogs when fed soy proteins (n=17) or soybean oil (n=1) or fecal dry matter of the dogs to evaluate the stool quality (n=18) (Table 4). Another manuscript by Kaur et al. (2021) conducted an *in vitro* digestibility trial for diets including soy nuggets. Among these 21 studies, 10 compared different types of soy ingredients (different anti-nutritional factor levels or differently processed ingredients) to each other or poultry meal, 9 studies evaluated the impact of different levels of inclusion of a certain soy-derived ingredient, and 2 studies evaluated the effects of adding exogenous enzymes into diets for dogs containing soy ingredients. There were 5 manuscripts (Hill et al., 2000; Hill et al., 2001; Yamka et al., 2003; Yamka et al., 2005a; Yamka et al., 2005b) that reported ileal (prececal) digestibility of experimental diets containing soy in dogs (Table 5). Three of them (Yamka et al., 2003; 2005a; 2005b) reported both ileal tract digestibility and apparent total tract digestibility (ATTD) of the diets. There were 4 manuscripts (Burkhalter et al., 2001; Sabchuk et al., 2017; Detweiler et al., 2019a; Detweiler et al., 2019b) that measured ATTD of the diets in dogs or cats when soy hulls were included in the experimental diets (Table 6). The remaining 5 studies of the 31 animal health and nutrition manuscripts didn’t measure either digestibility or stool quality but studied

the effect of soy on dogs' health by evaluating blood profiles, body condition, or skin condition of the fed animals, *in vitro* fermentation, or phytoestrogen content in the dogs' diets.

The nutrient digestibility comparison between soy proteins and poultry meal was inconsistent between the articles. One article found no difference in DM ATTD between soy protein fractions (soybean meal, soy flour, soy protein concentrates) and poultry meal (Clapper et al., 2001). Venturini et al. (2018) also reported no significant differences in the ATTD of DM among soy protein concentrate, maize gluten meal, and poultry by-product meal. Carciofi et al. (2009) reported that DM ATTD was higher ($P<0.05$) for micronized whole soybeans-containing diets than in the poultry by-product meal treatment. In contrast, one article observed a higher DM ATTD for poultry meal-containing diets than diets containing soy protein (Yamka et al., 2005a). When comparing CP ATTD of soy protein-containing diets to a poultry meal diet, the CP ATTD of soy protein diets was found to be higher than that of the poultry meal diet in Clapper et al. (2001), but no differences were found between CP ATTD of micronized whole soybeans and poultry by-product meal in Carciofi et al. (2009), and the CP ATTD for the poultry meal diet was higher than the soy protein-containing foods in Yamka et al. (2005a). In addition, there were no differences in amino acid ATTDs between soy protein fractions and poultry meal (Yamka et al., 2005a).

The researchers compared the nutrient digestibility in dogs of various types of soy protein ingredients, and those results varied as well. For example, there was no difference in DM ATTD of soybean meal and soya nuggets in homemade dog foods (Pawar and Pattanaik, 2009) or among soybean meal, red lentil, and green gram beans (Dhaliwal et al., 2016). The ATTD of DM was highest for soy protein isolate, followed by soybean meal and hydrolyzed soy protein concentrate, and lowest for soy protein concentrate (Félix et al., 2013b). The ATTD of DM was greater for low-phytate soybean meal than for SBM in Yamka et al. (2005b). The DM ATTD for soybean meal was higher than whole soybean (WSB) treatments (low

oligosaccharide WSB, low-oligosaccharide and low-phytate WSB) in Yamka et al. (2005a). However, micronized whole soybeans had a higher DM ATTD than SBM in Carciofi et al. (2009). Félix et al. (2013a) also studied micronized whole soybeans and corroborated that they had a higher DM ATTD than soybean meal along with defatted soybean meal, toasted soybeans, and raw soybeans in both adult dogs and growing puppies.

Similar to DM ATTD, CP ATTD comparisons among soy protein ingredients varied by study. One experiment found the ATTD of CP was highest for soy protein isolate, followed by soybean meal and hydrolyzed soy protein concentrate, and lowest for the soy protein concentrate (Félix et al., 2013b). The CP ATTD of soybean meal was found to be similar ($P > 0.05$) to that of red lentil and green gram in dogs fed twice daily (Dhaliwal et al., 2016; Pattanaik and Kore, 2021). Yamka et al. (2005a) reported higher CP ATTD of soybean meal than low-oligosaccharide whole soybeans and low-oligosaccharide and low-phytate whole soybeans, while Carciofi et al. (2009) reported no differences in CP ATTD between micronized WSB and SBM. Digestibility of CP was lower for SBM than soy nuggets, potentially due to the higher crude fiber content in the SBM diet (Pawar and Pattanaik, 2009). Amino acid digestibility was similar among treatments (low-oligosaccharide, low-phytate SBM, conventional SBM, low-oligosaccharide, low-phytate WSB, and conventional WSB) except for tryptophan and histidine (Yamka et al., 2005b). Tryptophan and histidine digestibilities were higher in WSB compared to low-oligosaccharide, low-phytate WSB (Yamka et al., 2005b). The ATTDs of several essential amino acids (isoleucine, phenylalanine, and tryptophan) in SBM were higher than the two WSB treatments (low-oligosaccharide WSB & low-oligosaccharide low-phytate WSB), whereas there was no difference in ATTD of nonessential amino acids (Yamka et al., 2005a).

As we compared the nutrient digestibility data when different inclusion levels of soy protein ingredients were fed to dogs, the DM and CP ATTD increased in some studies (Félix

et al. 2012; Menniti et al. 2014) with the increase of soy ingredients in formulas; however, those digestibilities decreased in a greater number of studies (Hill et al., 2001; Yamka et al., 2003; Beloshapka et al., 2016; Félix et al., 2020; Kaur et al., 2021) as soy inclusion level increased. The inclusion level above which the decrease of the nutrient digestibility began varied by studies (14%, Hill et al., 2001; linear decrease, Yamka et al. 2003; 48%, Beloshapka et al, 2016; linear decrease, Félix et al., 2020; 5%, Kaur et al. 2021). Marx et al. (2015) studied the effects of soybean oil as a fat source at different levels in dry extruded dog food. They reported the ATTDs of ether extract (EE) and gross energy (GE) were higher for the soybean oil than beef tallow-coated diets when the fat source inclusion level was 13% (Marx et al., 2015).

From both studies that evaluated the effects of supplementing soybean meals with exogenous enzymes on nutrient digestibility, supplemental β-mannanase (5 g/kg) had no effect ($P > 0.05$) on ATTD of DM and nitrogen (Yamka et al., 2006), and various combinations of protease, cellulase, pectinase, phytase, beta-glucanase, and xylanase also had no effect ($P > 0.05$) on ATTD of several nutrients (Tortola et al., 2013).

Four manuscripts that reported the effect of inclusion of soy hulls in diets for dogs on ATTD are presented in Table 6. All four studies reported lower DM digestibility (either ileal digestibility or ATTD) in the dogs and cats fed fiber-containing diets versus no fiber diets. Sabchuk et al. (2017) reported that the ATTD of DM, CP, AHEE, and GE linearly decreased in dogs, as inclusion of soy hulls increased (from 0 to 16%). The dogs and cats exhibited similar ($P > 0.05$) DM digestibilities when fed beet pulp- and soybean hull-containing diets (Burkhalter et al., 2001; Detweiler et al., 2019a; Detweiler et al., 2019b). Burkhalter et al. (2001) evaluated the effects of soybean hulls containing different ratios of insoluble: soluble fiber (I:S) on nutrient digestibility using ileally cannulated dogs. Ileal digestibility of DM and organic matter (OM) had quadratic effects as I:S increased, having the highest digestibility when I:S ratios

were highest (7.21 and 5.18) and when I:S was lowest (1.86), compared to when I:S were intermediate (2.65 and 3.17). Total tract digestibility of DM was not affected ($P > 0.05$) by I:S ratio among the soy hull treatments.

1.4.3.2 Soy ingredients and blood chemistry

Five studies evaluated blood chemistry along with nutrient digestibility in dogs fed soy ingredients (Pawar and Pattanaik, 2009; Carciofi et al., 2009; Tortola et al., 2013; Menniti et al., 2014; Pattanaik and Kore, 2021). There was no significant influence of feeding diets including either soybean meal or soy nuggets observed on the blood metabolic profile of the dogs in Pawar and Pattanaik (2009), with most of the parameters falling within the normal ranges. Menniti et al. (2014) also reported that all serum biochemical and hematological components were within normal physiological ranges for healthy, adult dogs when they were fed experimental diets containing soybean meal from 0 to 17%. Blood levels of hemoglobin and hematocrit in dogs did not change from the pre-experimental values when soybean meal was included in their diets (Pattanaik and Kore, 2021). According to Carciofi et al. (2009), postprandial blood incremental urea and urea peak concentrations of dogs fed micronized whole soybeans, soybean meal, and poultry by-product meal did not differ; however, time to urea peak was delayed in dogs fed the micronized whole soybeans diet. In contrast, the postprandial incremental urea and the maximum value of urea increment were higher for dogs fed SBM-based diets than for dogs fed poultry meal-based diets in the first experiment reported by Tortola et al. (2013). They found a quadratic reduction in the postprandial incremental urea with exogenous enzyme addition to the SBM diets in their second experiment (Tortola et al., 2013). According to Menniti et al. (2014) who evaluated the effect of dietary inclusion levels of soybean meal on dogs, they found quadratic responses for urea nitrogen and urea nitrogen:creatinine to increasing SBM inclusion, with peaks occurring when the diet contained 6% SBM. Still, those parameters remained within the reference range for normal adult dogs.

Scheraiber et al. (2016) evaluated the effects of dietary soybean hulls (0 or 16% inclusion) on the blood biochemical profiles and the body condition of dogs. The addition of soybean hulls (replacing corn) in the diet did not change the blood profiles; however, it decreased the deposition of lipids in subcutaneous tissue in dogs. Oh et al. (2019) evaluated the general health, blood lipid levels, and skin condition in dogs given a dietary soy lecithin supplement. They reported no changes ($P > 0.05$) in blood profiles but did find improvement in the amount of exercise and skin exfoliation, suggesting soy lecithin could be a nutraceutical based on the positive effect on the dogs' general health condition. They noted the necessity of further studies to establish the appropriate dose level and administration frequency of soy lecithin in dogs. Proot et al. (2009) fed low-protein diets with either soy protein isolate or dehydrated poultry meat protein as their main protein source to dogs diagnosed with congenital portosystemic shunts and evaluated their blood profiles to check liver function. Both low-protein diets showed improvements in the hepatic encephalopathy score, but the soy protein isolate diet group had lower plasma ammonia than the poultry diet, suggesting better support of liver function by SPI in dogs.

1.4.3.3 Soy ingredients on fecal fermentative characteristics

There were fewer studies (n = 9) that measured fecal pH or fermentative end products (Table 7) than studies which measured nutrient digestibility (n = 21) or fecal dry matter (n = 19) when dogs were fed soy protein ingredients (Table 4). Among the nine studies, six manuscripts reported the fecal ammonia concentration (Pawar and Pattanaik, 2009; Félix et al., 2013a; Tortola et al., 2013; Félix et al., 2013a; Beloshapka et al., 2016; Venturini et al., 2018), three manuscripts presented the fecal short-chain fatty acid concentration (Pawar and Pattanaik, 2009; Tortola et al., 2013; Beloshapka et al., 2016), and one manuscript documented the fecal branched-chain fatty acid, phenol, and indole concentrations (Beloshapka et al., 2016). There were four studies that measured fecal pH or fermentative end products when dogs

were fed soy hulls (Table 8) (Sabchuk et al., 2017; Myint et al., 2017; Detweiler et al., 2019a; Detweiler et al., 2019b).

Tortola et al. (2013) found that the inclusion of SBM in comparison to poultry meal decreased fecal dry matter and increased fecal output. Fecal acetate, propionate, and total SCFAs concentrations were higher when the dogs were fed SBM-containing diets than with the poultry meal diet, which indicated an increase in hindgut fermentation activity with the SBM treatment. When they added different kinds of exogenous enzymes to the soybean meal-containing diets in their second experiment, fecal acetate, propionate, total SCFAs, and lactate concentrations increased. They also observed higher fecal pH, and fecal ammonia concentrations in dogs consuming a poultry meal diet compared to those fed soybeans. Fecal ammonia is one factor responsible for foul fecal odor (Félix et al., 2010) and a lower fecal pH is also associated with increased hindgut fermentation, which supports normal functioning of the large bowel (Brambillasca et al., 2010). In addition, SBM consumption by the dogs had no effect on fecal bacteria composition (Tortola et al., 2013).

Pawar and Pattanaik (2009) reported more fecal lactate, acetate, propionate, and total SCFAs in dogs fed a soybean meal diet compared to a soya nugget diet, likely due to the higher crude fiber content in the soybean meal diet. There were no differences in the other measured fecal characteristics, including fecal score, fecal DM, fecal pH, and fecal ammonia concentration between the two treatments. The feces of the dogs fed the soy protein isolate-containing diet had higher pH and DM content, but those dogs produced less feces on a fresh matter basis than other dogs fed soybean meal or soy protein concentrates (Félix et al., 2013b). Fecal ammonia content was not influenced by the dietary soy protein ingredients (Félix et al., 2013b). In addition, Félix et al. (2013b) reported that dietary SBM resulted in the highest intestinal gas production, but there were no differences among the other dietary soy protein ingredients.

Beloshapka et al. (2016) reported the total dietary fiber content increased (5.70 to 13.13% on DM basis) in the dog diets as bioprocessed soy protein inclusion level increased from 0 to 48%. Fresh fecal DM was lower and fecal acetate, propionate, and total SCFA concentrations were greater for dogs fed the 24 and 48% soy protein treatments compared with dogs fed the 0% soy protein diet. Fecal output was greater for dogs fed the 48% soy protein treatment; however, fecal pH was not affected by dietary soy protein inclusion. Fecal ammonia, isovalerate, isobutyrate, total BCFA concentrations, phenol, and indole concentrations were lower for dogs fed 48% soy protein than the control. Phenols and indoles, like ammonia, can worsen fecal odor, and some evidence suggests they have a negative impact on intestinal health (Swanson et al., 2002).

When comparing the effect of soybean hulls to other fiber sources, fecal dry matter content of the dogs fed soybean hulls was lower than from the dogs fed sugarcane or cellulose, but higher than beet pulp (Sabchuk et al., 2017). However, intestinal gas score and intestinal gas production area, measured by radiographic images taken before and after the test diet was fed, were not influenced ($P > 0.05$) by dietary fiber sources (Sabchuk et al., 2017). Myint et al. (2017) compared the effects of soybean hull and cellulose supplementation on dogs. Dietary soybean hulls in dogs decreased fecal pH compared with cellulose, with higher fecal total SCFAs, acetate, butyrate and lactate concentrations (Myint et al., 2017). Detweiler et al. (2019a) also reported higher total fecal SCFA concentrations in dogs fed soybean hulls or beet pulp than dogs fed cellulose or a no fiber diet. The fecal indole and skatole concentrations in dogs fed the soybean hull diet were lower than the cellulose diet, while fecal ammonia concentration was unaffected (Myint et al., 2017). They also found that soybean hull supplementation led to a higher relative proportion of total lactobacilli, which can lower intestinal pH, in dogs' feces than cellulose supplementation (Myint et al., 2017). Detweiler et al. (2019b) evaluated the effect of dietary fiber sources on cats and found no differences in

fecal ammonia or total phenol and indole concentrations among treatments (no fiber, beet pulp, cellulose, and soybean hulls). In addition, cats fed beet pulp had a greater total fecal SCFA concentration, followed by soybean hull and no fiber, with the lowest for cellulose treatment.

In addition, Yamka et al. (2006) evaluated the flatulence and fecal odor metabolites of dogs fed low-oligosaccharide low-phytate soybean meal, conventional soybean meal, or poultry by-product meal diets with or without supplemental β-mannanase. They reported no difference in flatulence or fecal odor metabolites such as indoles, phenols, and volatile sulfur-containing compounds when measured by solid-phase microextraction procedure with gas chromatography, regardless of supplemental enzyme; however, the different dietary protein sources did affect the fecal odor metabolites but not the flatulence. Although dogs fed poultry by-product meal had low fecal output, the fecal odor metabolites excreted per day were greater than dogs consuming low-oligosaccharide low-phytate soybean meal or conventional soybean meal diets. These data suggest that dogs fed poultry by-product meal as dietary protein source had feces that contained more unpleasant odor components than soy protein-fed dogs (Yamka et al., 2006).

1.4.4 Soy ingredients on palatability

There were six studies that evaluated the palatability of soy ingredients using dogs (Pawar and Pattanaik, 2009; Félix et al., 2012; Beloshapka et al., 2016; Sabchuk et al., 2017; Venturini et al., 2018; Pattanaik and Kore, 2021), while there was one study that assessed the palatability in cats (Carciofi et al., 2009). Two studies assessed the palatability of the foods to the dogs using a 1–4-point scale (1 = ate an entire meal without hesitation, 4 = refused to eat), and the other five studies used the two-bowl method which measures preference of one food over another by presenting two foods to dogs and recording the total quantity of each food consumed. Pawar and Pattanaik (2009) did not find a significant difference ($P > 0.05$) between the dietary treatments (soybean meal or soya nugget inclusion) in palatability to dogs. Pattanaik

and Kore (2021) also reported the palatability score of the experimental diets (soybean meal, red gram, or lentil inclusion) using the same 1–4-point scale. The palatability score was similar ($P > 0.05$) among the treatments.

Félix et al. (2012) measured the palatability of dog diets using a pair-wise diet comparison for two consecutive days. They made six comparisons to evaluate the effects of enzymes and of the type and level of soybean meal on diet palatability. Dogs consumed more regular soybean meal-containing diets (either 15 or 30%) than the control, which contained higher poultry offal meal and maize with no soybean meal. The study also reported the 30% regular soybean meal diet with enzymes included was preferred over the control or 30% regular soybean meal diet without enzymes. Beloshapka et al. (2016) performed two-bowl tests once daily for two days in a row to evaluate the palatability of bioprocessed soy protein to dogs. Based on the intake ratios from the experiments, they reported that the optimal inclusion of the bioprocessed soy protein was 12% with greater consumption by the dogs compared to the 0% control. Sabchuk et al. (2017) evaluated the palatability of dog foods containing sugarcane, beet pulp, cellulose, and graded levels of soybean hulls using the pair-wise diet comparison method for two consecutive days. There were no differences in food preference between the tested diets (reference to 4% soybean hull, reference to 16% soybean hull, reference to 13.1% sugar cane, reference to 16% beet pulp, reference to 12.1% cellulose). Venturini et al. (2018) evaluated the palatability of dog diets containing poultry by-product meal, maize gluten meal, or soy protein concentrate using the two-bowl test method. Dogs preferred the poultry by-product meal over the maize gluten meal diets. There were no differences in preference between poultry by-product meal and soy protein concentrate diets or soy protein concentrate and maize gluten meal diets.

Carciofi et al. (2009) evaluated palatability of experimental foods to cats using the two-bowl test method on three consecutive days, comparing the relative consumption of two

diets (micronized whole soybeans or corn gluten meal inclusion). They found that the cats preferred the diet containing micronized whole soybeans over the maize gluten meal diet with a 2-fold greater consumption rate.

1.4.5 Soy ingredients on dog behavior

Sabchuk et al. (2014) evaluated dogs' behavior for 24 h, recording the frequency of occurrence for each behavior while feeding diets with or without soy hulls. There were no differences in the dogs' behavior with dietary soy hull inclusion. Similarly, Scheraiber et al. (2018) evaluated dog behavior after eating diets with or without soybean hulls (0 or 16%). They observed a reduction in scratching behavior and stereotypical behavior (repetitive regular movements) ($P < 0.10$) in animals fed a diet containing soybean hulls (Scheraiber et al., 2018).

1.4.6 Soy ingredients on allergenicity and immunology

There were six studies evaluating hydrolyzed soy protein on immunologic responses by challenged dogs. The work by Jackson et al. (2003) observed significant pruritus (itchy skin) after an oral challenge with soy protein but not with hydrolyzed soy protein. The soy and corn-specific serum IgE did not increase in dogs post challenge. Similarly, Serra et al. (2006) found a significant reduction in soy-specific IgE binding to the hydrolyzed soy protein than to the native soy protein in serum obtained from dogs with soy hypersensitivity. Puigdemont et al. (2006) observed no response to oral administration of hydrolyzed soy protein in dogs with soy hypersensitivity. Moreover, Biourge et al. (2004) reported that dogs diagnosed as having an adverse food reaction or a combined adverse food reaction and atopy showed a decrease of the pruritus score after 2 months of feeding the soy hydrolysate-containing diets. Vandresen and Farias (2018) also reported on the pruritus score and the Canine Atopic Dermatitis Lesion Index, and they observed that the hydrolyzed soy dog food was effective at partially reducing clinical signs of food-induced atopic dermatitis, while the homemade food group did not ($P > 0.05$) present improvements. In addition, Biourge and Fontaine (2004) reported that a soy

hydrolysate-based diet could significantly improve the clinical conditions, fecal score, pruritus score, and skin lesions of dogs suffering both from exocrine pancreatic insufficiency and skin disease.

Willis-Mahn et al. (2014) evaluated soy antigens in dry dog foods that have a "no soy" claim and veterinary therapeutic dry dog foods designed for food elimination trials using enzyme-linked immunosorbent assay (ELISA) testing. They detected a positive response for soy antigens in three of the four "no soy"-claiming diets and four of the seven veterinary therapeutic diets. They concluded that a veterinary therapeutic diet should be carefully chosen to treat soy food adverse reactions in dogs. Mikawa et al. (2021) investigated the effects of oral administration of a fermented soybean product, natto, on the cellular immune activity of dogs. They reported that dietary natto increased the cytotoxic activity of peripheral natural killer cells and the expression of TNF-α in peripheral blood mononuclear cells after an antigen stimulation in dogs. They concluded that dietary natto might be beneficial in augmenting cellular immune activity in dogs.

In addition, Cerundolo et al. (2004) determined the phytoestrogen content in commercial dog foods that contained soybeans or soybean fractions and foods without any soybean-related ingredients listed on the label. They found that most of the diets that included soy ingredients had detectable concentrations of phytoestrogens, which could have biological effects when ingested by dogs long-term. To explore that possibility, Cerundolo et al. (2009) evaluated the effect of dietary soy isoflavones on general health, adrenocortical and thyroid gland function in dogs. They fed a hydrolyzed soy isolate-based diet or the same diet without isoflavones, and most serum concentrations of hormones were not affected by diet. However, they concluded that feeding soy to dogs on a long-term basis may influence endocrine function due to the phytoestrogens, although more studies are needed to confirm or refute this supposition.

1.4.7 Soy ingredients on petfood processing application

There were four studies (Purushotham et al., 2007; Venturini et al., 2018; Kaur et al., 2021; Lyng et al., 2022) that addressed pet food processing attributes in their research findings and two (Félix et al., 2013a; Félix et al., 2020) that evaluated the impact of extrusion processing on antinutritional factors of soybean ingredients in dog diets.

Purushotham et al. (2007) attempted to optimize steam-conditioning and extrusion operations to inactivate anti-nutritional factors in soybeans for pet food applications. They demonstrated that urease activity and trypsin inhibitor levels decreased (2.0 and 50 mg/g to 0.1 and 5mg/g, respectively) as the extrusion temperature increased to 120°C. Extrusion of soybeans between 120 and 140°C did not affect major nutrient compositions but did improve nutritional value through the inactivation of antinutritional factors. Urease activity was reduced in all diets containing 30% soybean protein products (defatted soybean meal, soybean meal, micronized soybeans, toasted soybeans, and raw soybeans) after extrusion, but trypsin inhibitor activity was reduced only in the diets containing defatted soybean meal, soybean meal, and raw soybeans (Félix et al., 2013a). Urease and trypsin inhibitor activity in the diets increased with the inclusion of raw soybean up to 30% before and after extrusion (Félix et al., 2020). Félix et al. (2020) also reported a decrease in antinutritional factor activity after extrusion. Kaur et al. (2021) prepared dog food using soy nuggets with three processing methods: raw, boiled, and extruded, and then measured the in vitro digestibility of nutrients. They concluded that the extrusion improved the digestibility of dry matter, crude protein, ether extract, and organic matter.

Venturini et al. (2018) evaluated the effect of soy protein concentrate at different inclusion levels up to 45% on extrusion processing and kibble macrostructure. The substitution of poultry by-product meal by coarse soy protein concentrate increased extrusion motor load, temperature, die pressure, and specific mechanical energy (SME). The bulk density of the

kibble, specific length, and radial expansion rate after dryer decreased, whereas the starch gelatinization increased with the increase of coarse soy protein concentrate in the dog diets. In summary, soy protein concentrate demonstrated good functionality during the extrusion processing and improved kibble expansion and starch gelatinization.

Lyng et al. (2022) investigated the effect of independent extrusion process variables when producing pet food extrudates containing defatted soy flour alone or combined with beef meat or connective tissue protein (collagen fiber). They found that defatted soy flour with water expanded less after extrusion and could not retain a chunk-like appearance after retorting. However, the defatted soy flour combined with beef meat or connective tissue expanded more and retained its pre-retort paste-like structure after retorting. Overall, they indicated that a combination of formula and extrusion process parameters have a significant effect on the extrusion processing and the resulting product characteristics.

1.5 Discussions

1.5.1 The strengths of soy in pet food

Soybeans should be described beyond their nutritional chemical composition to represent their value. Based on the summarized literature, the strengths of soybeans in pet food applications are nutritional, palatable, and functional processing attributes. Macronutrients in soybeans, such as fat, protein, and fiber, are either digestible or fermentable for companion animals, and the nutrient profiles are comparable to poultry by-product meal. Additionally, the variety of soybean ingredients with different chemical or physical characteristics enables formulators to increase or decrease certain nutrient digestibility, fermentability, or expansion of the processed final products.

The nutritional compositions of various soy ingredients discussed in the literature review can be explained by the usual soy protein processing stream. The soy protein processing starts with dehulled full fat soybeans, then they are defatted by oil extraction with hexane as

the solvent (Alden, 1975). To remove the remaining solvent from the defatted soybeans, various processing conditions in terms of heat temperature, moisture, and retention time can be used and have effects on protein denaturation related to activity of proteinaceous anti-nutritional factor activities (Van Krimpen et al., 2013). The results clearly showed that soybeans contain higher fat and energy density compared to soybean protein ingredients, which are made from the defatted soybean flakes. In addition, raw soybeans contain higher urease activity and trypsin inhibitors than soybean meal varieties or toasted soybeans. Defatted soybean flakes are used to make soybean meals, soy protein concentrate, soy protein isolate, hydrolyzed soy protein, or textured soy protein by applying different processing conditions (Alden, 1975). Among soy protein ingredients, soybean meal varieties have lower protein levels than soy protein concentrate varieties because the soluble carbohydrates are extracted from defatted flakes before grinding when making SPC. The protein dispersibility was higher for raw soybeans and soy protein isolate than micronized or toasted soybeans, soybean meal, or soy protein concentrates. Protein soluble in KOH solutions was also higher for raw soybeans and soy protein isolate than soy protein concentrates or soybean meal. Soybean processing influences the protein fraction, and the extent of the soy protein denaturation affects the protein digestibility (Van Krimpen et al., 2013). Félix et al. (2013b) reported the high correlations among CP digestibility, protein dispersibility index, and soluble protein contents in KOH. An unfolded protein structure can be more accessible to proteolytic digestive enzymes; however, it can also increase protein aggregation by increasing the interaction between protein with other proteins or components, which can lower accessibility to enzymes (Van Krimpen et al., 2013). Even though the various soy antinutritional factors and protein denaturation also impact the protein quality of the ingredients, only few studies have analyzed and published data on these effects in companion animals.

Numerous researchers have explored soy for its bioavailability in dogs and cats by measuring nutrient digestibility, stool quality, blood chemistry, and fecal fermentative characteristics. Evaluation of the effect of dietary soy ingredients on nutrient digestibility and stool quality has been the primary area of interest with the largest number of manuscripts published. Nutrient digestibility of soy proteins was comparable to poultry meal, often demonstrating no significant difference in the results of these studies. Because soy protein fractions used in experiments differ from soy flour to conventional soybean meal to soy protein concentrate, it is hard to state which has better nutrient digestibility between soy proteins vs. poultry proteins, but most are at least comparable. Nutrient digestibility among various soy protein ingredients varied based on their preparation/processing, anti-nutritional factor concentrations, and crude fiber contents. The nutrient digestibility of dog diets was reported to decrease with increasing inclusion levels of soy protein ingredients in more manuscripts than increase or remain the same.

None of the studies included in this review found changes in blood chemistry beyond accepted reference values due to dietary soy ingredients in dogs, showing no deleterious effects on animal health in that regard. Some studies found fecal production increased with inclusion of dietary soybean meal compared to poultry meal due to the higher fiber content, but it also resulted in higher fecal fermentative products such as SCFA. In addition, putrefactive compounds in feces such as indole, skatole, and ammonia were either no different or lower in dogs fed soy ingredients than beet pulp or no fiber. This high fermentability of soy ingredients in dogs impacts the gut microbiota population and is beneficial for their gut health. Soybean ingredients had either no effect on or increased the palatability of pet foods in animals, which is promising for pet food formulators.

Soy protein is known for excellent functional properties, such as water holding, gelling, fat absorbing, and emulsifying capacities in food products, which is why it is currently

used as an ingredient in extrusion (Ismail et al., 2020). Inclusion of soy ingredients influenced the extrusion processing parameters and the expansion of the dog kibbles (Venturini et al., 2018). However, there was no significant negative effect on the kibble formation, which is one of the critical factors for industrial producers when choosing an ingredient for their formulas. Further studies to compare the processing functionality among various soy ingredients or comparing soy to other commonly used animal or plant proteins would be helpful to demonstrate the strength of the soybean in pet food processing.

1.5.2 The weaknesses of soy in pet food

The weakness of dietary soybeans for companion animals lies in their antinutritional factors and potential allergenicity. However, there are no routine tests in normal feed use to detect antigenic or toxic activity of soybean components unless these are monitored separately (Csaky and Fekete, 2004). Furthermore, no data are available on the variability of antigenic components between soybean varieties, source of the soybeans, or various soy ingredients. Possible pathological effects of dietary soybean on various animals (rats, piglets, and. preruminant calves) were indicated by Csaky and Fekete (2004). They reported that antinutritive factors such as trypsin inhibitors induced pancreas hypertrophy, lowered methionine and cysteine absorption, shortened villi in the small intestine, and reduced growth performance in animals (Csaky and Fekete, 2004). Soy oligosaccharides are considered antinutritional factors that may induce flatulence in dogs (Silvio et al., 2000). The intestinal gas production in dogs fed soybean meal was higher, but there was no difference when comparing the reference diet to soy protein concentrate or soy protein isolate (Félix et al., 2013b). In addition, the other study that examined this outcome found no differences in flatulence among low-oligosaccharide low-phytate soybean meal, conventional soybean meal, and poultry by-product meal (Yamka et al., 2006).

Dréau and Lallès (1999) reported that the predominant storage proteins of soybeans, glycinin and β-conglycinin, are antigens and can cause allergenic reactions in the intestinal mucosa of preruminant calves and early weaned piglets. All subunits from the soybean glycinin protein family were identified as soybean allergens for humans (Helm et al., 2000). Fu et al. (2007) further identified soybean β-conglycinin α-subunit as a potential allergen for young piglets. Taliercio et al. (2014) identified the β-subunit of soybean β-conglycinin as antigenic in dogs by demonstrating the data that peptides of the β-subunit of conglycinin were bound by IgG and IgE antibodies from canine' sera. However, the pathological effects on companion animal health and immunological responses to dietary soybean or SBM need to be studied further.

Various approaches such as food processing technology, genetic engineering, and targeted breeding have been studied to remove antinutritional factors and allergens from soybeans (Fu et al., 2007). The activities of antinutritional factors and allergens in soy can be adjusted by enzyme, heat, ethanol treatment, or fermentation, although most treatments leave conglycinin residues intact (Cervantes-Pahm and Stein, 2010; Herkelman et al., 1992; Rickert, 2003; Matsumoto et al., 2019; Kiers et al., 2003; Csaky and Fekete, 2004). To lower adverse food reactions such as food allergy in dogs, soybean meal is often hydrolyzed and used for hypoallergenic prescription diets. The literature shows that hydrolyzed soy protein has significantly lower allergenic reactions compared to soy or soybean meal, supporting the supposition that hydrolysis of soy proteins overcomes the weaknesses of soy in pet food application and provides new opportunities (Jackson et al., 2003; Puigdemont et al., 2003; Biourge et al., 2004; Vandresen and Farias, 2018). For further research, it would be valuable to compare allergenicity between soy proteins and other animal proteins. This is because the most common food allergens in dogs with diagnosed food allergies are beef, dairy, or chicken, while the soy is one of the least common food allergens in dogs (Mueller et al., 2016). Hot

water treatment, aqueous alcohol extraction, or isoelectric protein precipitation used to manufacture soy protein concentrates and isolates remove the oligosaccharides (Rackis, 1981), resulting in lower flatus activity. The optimal processing conditions to remove or reduce all soy antinutritional factors, such as trypsin inhibitors, urease, lectins, conglycinin, oligosaccharides, and overall antigenicity, and increase the protein quality should be assessed to deliver the maximal nutritional value of soybeans to pet animals. Again, the selection of the correct soy ingredient among various choices can optimize the value of soy ingredients.

1.5.3 The opportunities of soy in pet food

As the market matures and premiumization progresses, pet food development has focused on functional health benefits and sustainability in recent years. Opportunities for soybeans are increasing with the ongoing pet food trends. The pet food industry produces more segmented products making claims such as ‘gut health’, ‘vegetarian’, ‘vegan’, ‘plant-based’, and ‘sustainable dog food’ to fit the specific needs of consumers. About 20 million vegetarian pet owners are in the United States, and 45% of pet owners (including non-vegetarian pet owners) expressed a desire to feed a plant-based diet if one were available that met their criteria (Dodd et al., 2019). There is a small but growing niche market for vegetarian pet foods. Soy has been researched because it offers high protein and a good amino acid composition similar to that of meat (Brown, 2009). Soy protein sources had lower methionine than poultry meal (Clapper et al., 2001), which may necessitate supplemental methionine, cystine, and taurine in the formulas to meet the amino acid requirements for dogs and cats. A recent study by Golder et al. (2020) reported no differences in digestibility between plant and animal protein in dogs and found that the plant protein had higher digestibility than animal protein in cats. However, soybean products need to be adequately processed to achieve equal nutrient digestibility to meat proteins in dogs (Kanakubo et al., 2015).

Obesity in dogs has been identified as a pressing issue that may negatively affect animal health and longevity (Bland et al, 2009). Thirty-nine percent of dog owners and 45% of cat owners reported that their pets are overweight or having obesity (Association for Pet Obesity Prevention, 2021). Numerous factors such as genetics, amount of physical activity, and the energy consumption from their diet or treats are involved in pets becoming obese (German, 2006). To lose weight, feeding high-fiber diets has been studied and was shown to induce weight loss and satiety in dogs (Weber et al., 2007; Fritsch et al., 2010). Inclusion of soy hulls as fiber in diets led to lesser nutrient digestibility according to this literature review, and this can dilute energy consumption from diets and increase weight loss in animals. Dietary soybean hulls decreased the deposition of subcutaneous lipids in dogs (Scheraiber et al., 2016), providing further evidence that soy ingredients could benefit animal health. Studies on dog behavior versus dietary soy hull inclusion showed either no difference or a reduction of scratching behavior with lower metabolizable energy intake when soy hulls were present in the diet (Sabchuk et al., 2014; Scheraiber et al., 2018). Numerous studies in the literature showed that dietary soy ingredients increased fermentative products by the hindgut microbiome and can result in a healthy gut by acting as prebiotics in companion animals. Addition of exogenous carbohydrase enzymes to the diets was not effective at increasing nutrient digestibility in dogs according to two manuscripts. However, a carbohydrase mixture did show improvements to nutrient digestibility in pigs (Kim et al., 2003; Kim et al., 2006; Ao et al., 2010; Ayoade et al., 2012), which supports the potential for improved digestibility through enzyme supplementation in dogs. Traylor et al. (2001) reported that phytase supplementation improved Ca and P utilization from soybean meal by growing swine. Kerr et al. (2010) also reported clear improvements in P digestibility in finishing pigs when fed phytase with soybean meal diets. Further research is needed to find out the right enzymes and enzyme supplementation timing for dogs to increase the nutrient utilization of soybeans. In addition, microbially

fermented soy ingredients and phytoestrogens, such as isoflavones, were suggested to have additional potential health benefits for dogs (Cerundolo et al., 2009; Mikawa et al., 2021). More detailed research about optimal dose and health effects from long-term consumption of these functional soy-based ingredients may provide more opportunities to expand soy utilization in pet food. In addition, there is an obvious need to conduct more studies in cats, since information regarding effects of dietary soy ingredients on cat nutrition or health was scarcer than dogs in literature.

1.5.4 The threats of soy in pet food

The predominant threat to the use of soybeans is the underlying negative perceptions of soy among some marketers and consumers. One of the top pet food marketing claims in 2021 was "no corn or soy," which influenced 25% of pet owners' decisions to purchase specific pet food according to the survey conducted by the Association for Pet Obesity Prevention (Association for Pet Obesity Prevention, 2021). According to another survey (Association for Pet Obesity Prevention, 2018), pet owners were more influenced by the descriptive statements about diets when purchasing pet foods versus veterinary professionals. These two surveys support the idea that any statements about soy would cause more concern among owners than professionals, indicating the potential for misinformation to sway owners or a disconnect with the science. There are several online articles that are approachable to consumers due to their language and style that assert that soybeans are bad for dogs. Some of the reasons they provide for avoiding soy in dog foods are 1) because most U.S.-grown soybeans are genetically modified organisms (GMO) that contain glyphosate resistance, 2) soy contains antinutrients such as lectins that can cause digestive issues and may lead to leaky gut syndrome, 3) soy may lower thyroid function by goitrogenic effects, 4) soy may trigger food allergies, 5) phytoestrogens in soy are potentially harmful and can lead to infertility, polycystic ovarian syndrome and breast cancer, 6) soy is antigenic, 7) soy contains trypsin inhibitors, 8) soy is

high is phytic acid, etc. (Henriques, 2022). However, most of the reasons are assumptions with no published scientific evidence. The results of this systematic review regarding dietary soy ingredients rather supports the positive nutritive value of soy for companion animals. With the expansion of press reporting on and disseminating scientific research data in recent years, there are some consumer-friendly sources that try to educate consumers on the facts about soy, including that it has high nutritional value and is well-digested in dogs. One such article explains that there is no evidence that normal levels of soy in dog foods can lead to illness (Soy in dog food: what you need to know | American kennel club). Bioactive proteins such as trypsin inhibitors or lectins in soy are denatured by the cooking process (Riaz, 2000) and they should not be an issue in conventional processed pet foods. Pet food companies themselves, such as Purina, are attempting to address the challenge of misinformation (Myth or Fact? Soy is an undesirable pet food ingredient. | Purina MythBusters Soy). They counter several common myths, such as explaining that soybean meal does not increase flatulence or bloat in dogs (Davenport et al., 2000; Yamka et al., 2006) and that SBM is not highly allergenic (Verlinden et al., 2006). These efforts could start to shift the perception of soy among consumers and producers, potentially making them more open-minded. The recent white paper on soy in pet foods from ADM revealed that 80% of U.S. pet owners are open to soy, corn or wheat in their pets' diet by conducting independent consumer surveys (ADM, 2022). According to the survey, motivators for including soy were health/nutrition, taste, and recommendations. Another white paper published by ADM also supported that pet parent attitudes about soy ingredients for their pets are highly open-minded, suggesting that the influence of the "no corn, soy or wheat" slogan that has impacted the pet food industry for nearly three decades is running out (ADM, 2021). Because pet foods are commercial products, the perception of consumers and producers and marketing slogans can significantly affect the utilization of soy. Therefore, scientists must conduct studies to investigate the negative claims of soy in pet foods and publish

studies to set straight the myths of incorrect information about soy in pet foods. With the research publications in scientific journals and online magazines, periodic surveys that examine the general perception of soy are also necessary to 1) determine if the scientific evidence has been communicated well to industrial stakeholders and consumers, not just with other scientists, and 2) predict the future market needs for soy.

1.6 Conclusion

This paper comprehensively summarizes the effects of various soy ingredients in multiple pet food applications. It provides an overview of gaps in the research where more attention is needed from future researchers in the pet food industry. Various food processing technologies have been applied to soybeans to produce ingredients that contain desired nutrients in higher concentrations. The versatility of soybean ingredients has been demonstrated to offer considerable potential for inclusion as oil, protein, fiber, or functional ingredients in pet food. Questions remain regarding the concentrations of antinutritional factors in various soy ingredients and efficient pet food processing conditions or exogenous enzyme supplementation methods to completely overcome concerns about these factors. More feeding trials on pet food processing of prepared diets with soy ingredients are required to determine the relationship between processing and the nutritional value of the diets. More research is needed on the potentially beneficial effects of hindgut fermentation and functional fractions of dietary soy. Lastly, research that studies the effect of inclusion of soy ingredients in pet foods on human-pet owners' perceptions, such as consumer studies, sensory analysis, or survey studies, will be needed to better understand soy's acceptability and overcome any barriers in the future pet food industry.

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1.8 Chapter 1 Figures

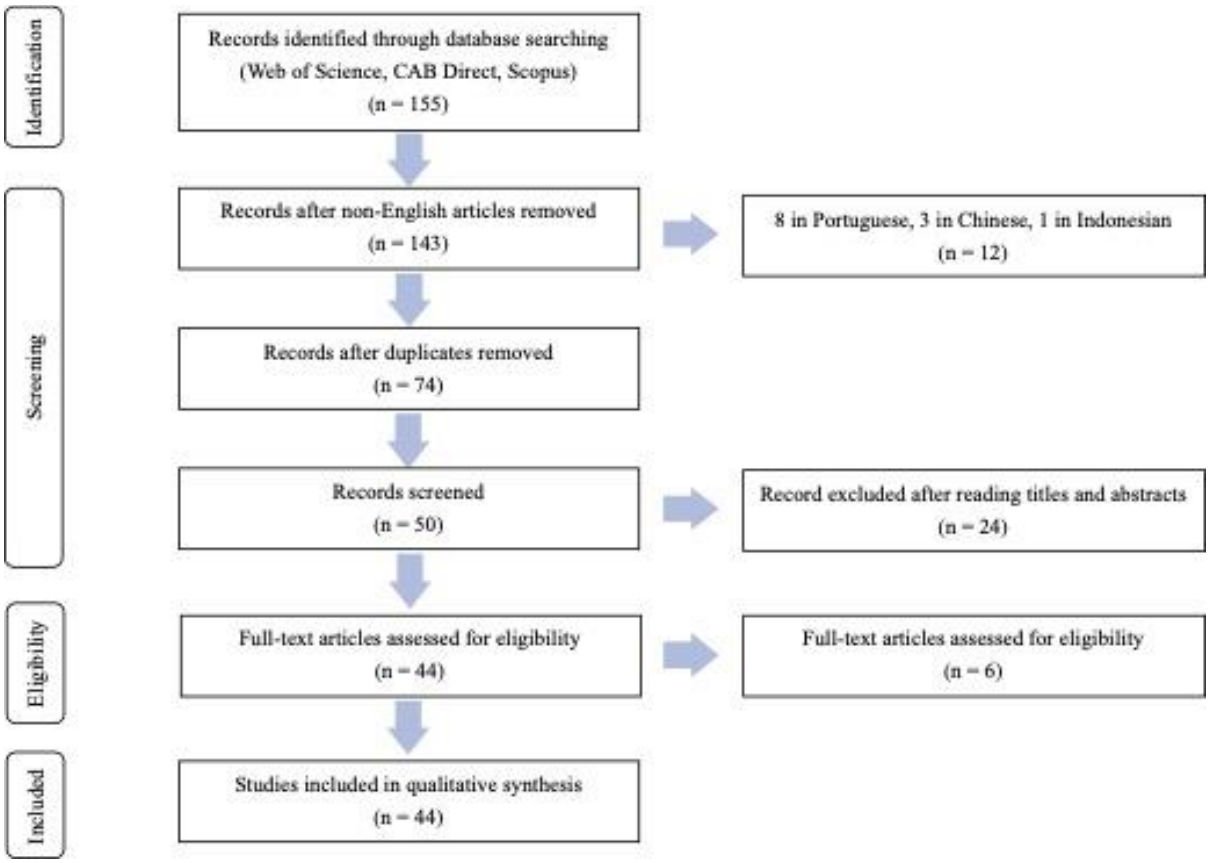

Figure 1.1Prisma flow diagram that identifies the total number of articles initially surveyed, the number of articles included and excluded for this systematic review.

1.9 Chapter 1 Tables

Table 1.1 Nutritional composition (g/kg on dry matter, unless otherwise stated) of soy ingredients in various pet food studies

Authors	Soy	[1]DM	Ash	[2]CP	[3]AHEE	[4]GE, MJ/kg	Ca	Total P	Crude fiber	[5]NDF	[6]ADF	[7]TDF
Clapper et al., 2001	Soybean meal	874	74	566	25	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	157
Clapper et al., 2001	[10]Soy flour	927	70	553	28	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	162
Clapper et al., 2001	[11]Soy protein concentrate 1	949	61	722	11	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	213
Clapper et al., 2001	[12]Soy protein concentrate 2	943	70	704	8	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	175
Clapper et al., 2001	[13]Soy protein concentrate 3	945	41	705	32	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	211
Yamka et al., 2006	Low-oligosaccharide low-phytate soybean meal	967.5	47	197.6	115	n.r.	8	7	23	n.r.	n.r.	n.r.
Yamka et al., 2006	Soybean meal	970.1	48	204.1	117	n.r.	8	7	27	n.r.	n.r.	n.r.
Carciofi et al., 2009	Soybean meal	861.9	34.8	479	21.9	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Carciofi et al., 2009	Micronized whole soybeans	938.3	44.6	412.5	250.5	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Félix et al., 2012	Regular soybean meal	898.8	71.2	515.1	45.1	19.8	n.r.	n.r.	73.2	n.r.	n.r.	n.r.
Félix et al., 2012	High-protein soybean meal	891.5	68.2	561.2	45.9	20.2	n.r.	n.r.	51.7	n.r.	n.r.	n.r.
Félix et al., 2013a	Defatted soybean meal	939.1	61.4	524.1	26.2	19.5	4	9	16	89.1	n.r.	n.r.
Félix et al., 2013a	Soybean meal	892.2	61	467.2	41.1	20.2	4	6	53.4	147.2	n.r.	n.r.
Félix et al., 2013a	Micronized soybeans	957.3	47	408	215.4	24.2	2	6	15.3	139	n.r.	n.r.
Félix et al., 2013a	Toasted soybeans	897.1	49.8	376.2	234	23.8	3.1	6.1	41.3	106	n.r.	n.r.
Félix et al., 2013a	Raw soybeans	895	49	376.4	231.2	23.7	3.1	6.2	40.5	104.2	n.r.	n.r.

Félix et al., 2013b	Soybean meal	902.3	62.6	556.1	36.6	20.5	5.4	6.8	56.1	170.8	85.3	n.r.
Félix et al., 2013b	[14]Soy protein concentrate	911.1	64.5	618.8	21.5	20.7	6.6	9	45.2	271.4	85	n.r.
Félix et al., 2013b	[15]Soy protein concentrate	917.3	60.5	687.2	18.5	20.7	5.2	9.1	46.4	282	87.8	n.r.
Félix et al., 2013b	[16]Hydrolyzed soy protein concentrate	906.9	61.8	692.2	19.8	20.7	5.8	9.2	47.9	292.5	87.4	n.r.
Félix et al., 2013b	Soy protein isolate	955.9	45.1	898	36.9	23.2	3.3	7.1	0.6	32.2	8.5	n.r.
Menniti et al., 2014	Soybean meal	881	68.1	552.8	35.2	n.r.	3.9	5.9	37.5	n.r.	n.r.	n.r.
Venturini et al., 2018	[17]Soybean concentrate	927	74.4	673.1	4.3	n.r.	n.r.	n.r.	49.6	n.r.	n.r.	n.r.
Venturini et al., 2018	[18]Soybean concentrate	940	81.9	643.6	4.3	n.r.	n.r.	n.r.	30.9	n.r.	n.r.	n.r.
Félix et al., 2020	Raw soybeans	907.2	45.5	398.3	207.2	24.1	n.r.	n.r.	69.7	n.r.	n.r.	n.r.

[1]DM = dry matter, [2]CP = crude protein, [3]AHEE = acid hydrolyzed ether extract, [4]GE = gross energy, [5]NDF = neutral detergent fiber, [6]ADF = acid detergent fiber, [7]TDF = total dietary fiber, [8]IF = insoluble fiber, [9]SF = soluble fiber, [10]Soy flour = Soyafluff 200W, [11]Soy protein concentrate = traditional aqueous alcohol-extracted soy protein concentrate (Profine F), [12]Soy protein concentrate 2 = extruded soy protein concentrate (Profine E), [13]Soy protein concentrate 3 = modified molecular weight soy protein concentrate (Soyarich I), [14]Soy protein concentrate = soy protein concentrate with 600 g crude protein/kg, [15]Soy protein concentrate = soy protein concentrate with 700 g crude protein/kg, [16]Hydrolyzed soy protein concentrate = soy protein concentrate with 700 g crude protein/kg, [17]Soybean concentrate = coarse particle size, [18]Soybean concentrate = small particle size; 200 μm.

Table 1.2 Anti-nutritional factors, protein dispersibility index (PDI), and sugar compositions (dry matter basis, unless otherwise stated) of soy ingredients in various pet food studies

Authors	Soy	Urease, Δ pH	Typsin inhibitor, mg/g	Phytate, g/kg	Stachyose, g/kg	Raffinose, g/kg	Sucrose, g/kg	Galactose, g/kg	Fructose, g/kg	Total sugar, g/kg	PDI, %	Protein soluble in KOH, %
Yamka et al., 2006	Low-oligosaccharide low-phytate soybean meal	n.r.	n.r.	0.7	0.1	0.1	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Yamka et al., 2006	Soybean meal	n.r.	n.r.	1.5	22.4	2	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Purushotham et al., 2007*	Raw soybeans	2	51	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Félix et al., 2012*	Regular soybean meal	0.05	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	10.7	n.r.
Félix et al., 2012*	High-protein soybean meal	0.04	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	10.3	n.r.
Félix et al., 2013	Defatted soybean meal	0.22	9	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	8.56	n.r.

Félix et al., 2013	Soybean meal	0.05	6.6	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	10.74	n.r.
Félix et al., 2013	Micronized soybeans	0.04	6.6	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	13.03	n.r.
Félix et al., 2013	Toasted soybeans	0.07	3.1	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	10.31	n.r.
Félix et al., 2013	Raw soybeans	1.74	45.1	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	54.24	n.r.
Félix et al., 2013	Soybean meal	0.01	n.r.	n.r.	47.5	26.3	88.3	12.1	0.8	175	24.01	68.05
Félix et al., 2013	[1]Soy protein concentrate	0.01	n.r.	n.r.	24.4	10.3	24.6	0	0	59.2	11.01	42.35
Félix et al., 2013	[2]Soy protein concentrate	0.01	n.r.	n.r.	4.8	1.9	4.8	0	0	11.4	16.69	56.61
Félix et al., 2013	[3]Hydrolyzed soy protein concentrate	0.03	n.r.	n.r.	4.5	2	4.3	0	0	10.8	21.7	66.02
Félix et al., 2013	Soy protein isolate	1.52	n.r.	n.r.	0.1	0	0.3	0	0	0.4	43.52	87.41
Félix et al., 2020*	Raw soybeans	1.86	15.91	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	89.3

[1]Soy protein concentrate with 600 g crude protein/kg, [2]Soy protein concentrate with 700 g crude protein/kg, [3]Hydrolyzed soy protein concentrate with 700 g crude protein/kg.

*Values were reported on as-is basis.

Table 1.3 Nutritional composition (g/kg on dry matter, unless otherwise stated) of soy hulls in various pet food studies

Authors	Soy hull	DM[1]	Ash	CP[2]	AHEE[3]	Crude fiber	NDF[4]	ADF[5]	TDF[6]	IF[7]	SF[8]
Burkhalter et al., 2001	Soybean hulls (Cargill)	913	53	92	n.r.	n.r.	n.r.	n.r.	773	700	73
Burkhalter et al., 2001	Soybean hulls (Central Soya)	920	49	123	n.r.	n.r.	n.r.	n.r.	807	722	85
Burkhalter et al., 2001	Soybean hulls (Jones A)	913	51	130	n.r.	n.r.	n.r.	n.r.	764	637	127
Burkhalter et al., 2001	Soybean hulls (Jones B)	913	57	149	n.r.	n.r.	n.r.	n.r.	755	684	71
Burkhalter et al., 2001	Soybean hulls (Quincy)	947	53	155	n.r.	n.r.	n.r.	n.r.	638	599	39
Sabchuk et al., 2014	Soya hulls (as-fed basis)			130	n.r.	n.r.	n.r.	n.r.	720	655	65
Sabchuk et al., 2017	Soya hulls	897.9		130.1	58.8	384.5	834.8	n.r.	720.8	655.1	65.7

[1]DM = dry matter, [2]CP = crude protein, [3]AHEE = acid hydrolyzed ether extract, [4]NDF = neutral detergent fiber, [5]ADF = acid detergent fiber, [6]TDF = total dietary fiber, [7]IF = insoluble fiber, [8]SF = soluble fiber

Table 1.4 Apparent total tract digestibility and fecal dry matter (%) in companion animals fed soy included diets

Authors	Soy	Inclusion, %	Animal	Apparent total tract digestibility, %							Fecal DM, %
				[1]DM	[2]OM	[3]CP	[4]AHEE	[5]GE	[6]TDF	[7]CF	
Clapper et al., 2001	soybean meal	44.03	Adult dog	81.8	81.7	83.9	92.5	83.8	n.r.	n.r.	n.r.
Clapper et al., 2001	soy flour (Soyafluff 200W)	45.16	Adult dog	79.6	85.6	87.3	95.5	87.8	n.r.	n.r.	n.r.
Clapper et al., 2001	SPC 1 (Profine F)	33.17	Adult dog	79.8	84.4	86.5	93.3	86.2	n.r.	n.r.	n.r.
Clapper et al., 2001	SPC 2 (Profine E)	34.06	Adult dog	82.2	84.3	84.7	93.7	86	n.r.	n.r.	n.r.
Clapper et al., 2001	SPC 3 (Soyarich I)	33.99	Adult dog	80.9	86.8	89.3	94.5	88.5	n.r.	n.r.	n.r.
Clapper et al., 2001	Poultry meal	32.76	Adult dog	81.9	84.7	76.9	92.9	84.9	n.r.	n.r.	n.r.
Yamka et al., 2003	soybean meal	15.1	Adult dog	83.1	n.r.	68.1	n.r.	n.r.	n.r.	n.r.	61.1
Yamka et al., 2003	soybean meal	25.5	Adult dog	75.7	n.r.	68.6	n.r.	n.r.	n.r.	n.r.	67.2

Yamka et al., 2003	soybean meal	36.0	Adult dog	64.4	n.r.	64.3	n.r.	n.r.	n.r.	n.r.	70.3
Yamka et al., 2003	soybean meal	46.1	Adult dog	57.4	n.r.	65.5	n.r.	n.r.	n.r.	n.r.	73.5
Yamka et al., 2005a	low-oligosaccharide whole soya beans	40.12	Adult dog	85.9	n.r.	81.8.	n.r.	n.r.	n.r.	n.r.	33.8
Yamka et al., 2005a	low-oligosaccharide low-phytate whole soya beans	45.21	Adult dog	85.4	n.r.	82.4	n.r.	n.r.	n.r.	n.r.	28.8
Yamka et al., 2005a	soya bean meal	31.73	Adult dog	89.1	n.r.	84.8	n.r.	n.r.	n.r.	n.r.	28.5
Yamka et al., 2005a	Poultry meal, low-ash	22.38	Adult dog	91.3	n.r.	86.4	n.r.	n.r.	n.r.	n.r.	48.9
Yamka et al., 2005b	low-oligosaccharide, low-phytate soybean meal	29.22	Adult dog	87.0	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	35.5
Yamka et al., 2005b	conventional soybean meal	30.85	Adult dog	84.8	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	36.4

Yamka et al., 2005b	low-oligosaccharide, low-phytate whole soybean	45.25	Adult dog	82.7	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	35.9
Yamka et al., 2005b	conventional whole soybean	40.1	Adult dog	83.8	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	36.3
Yamka et al., 2006	Low os low phytate soybean meal + β-mannanase (5 g/kg)	29.26	Adult dog	88.0	n.r.	85.1	n.r.	n.r.	n.r.	n.r.	32.3
Yamka et al., 2006	Low os low phytate soybean meal	29.26	Adult dog	88.0	n.r.	85.6	n.r.	n.r.	n.r.	n.r.	29.8
Yamka et al., 2006	soybean meal + β-mannanase (5 g/kg)	30.93	Adult dog	86.5	n.r.	85.2	n.r.	n.r.	n.r.	n.r.	29.3
Yamka et al., 2006	soybean meal	30.93	Adult dog	85.5	n.r.	84.5	n.r.	n.r.	n.r.	n.r.	29.4
Yamka et al., 2006	Poultry by-product meal + β-	22.43	Adult dog	91.3	n.r.	86.9	n.r.	n.r.	n.r.	n.r.	40.74

	mannanase (5 g/kg)										
Yamka et al., 2006	Poultry by-product meal	22.43	Adult dog	91.3	n.r.	86.8	n.r.	n.r.	n.r.	n.r.	39.57
Pawar and Pattanaik, 2009	soybean meal	50	Adult dog	84.2	86.2	94.0	87.7	n.r.	n.r.	37.1	27.1
Pawar and Pattanaik, 2009	soya nugget	50	Adult dog	86.0	87.0	96.1	91.6	n.r.	n.r.	63.7	23.8
Carciofi et al., 2009	micronized whole soybean	29.3	Adult cat	82.0	85.0	84.0	90.0	86.0	n.r.	n.r.	31.1
Carciofi et al., 2009	Corn gluten meal	17.2	Adult cat	81.0	85.0	84.0	86.0	85.0	n.r.	n.r.	30.9
Carciofi et al., 2009	micronized whole soybean	33.5	Adult dog	86.0	89.0	87.0	94.0	89.0	n.r.	n.r.	30.9
Carciofi et al., 2009	soybean meal	29.5	Adult dog	84.0	88.0	86.0	92.0	89.0	n.r.	n.r.	31.8
Carciofi et al., 2009	Poultry by-product meal	22.8	Adult dog	83.0	88.0	85.0	92.0	89.0	n.r.	n.r.	45.4
Hill et al., 2001	texturized soy protein	0	Adult dog	87.0	n.r.	86.3	98.9	92.4	n.r.	n.r.	39.0
Hill et al., 2001	texturized soy protein	14	Adult dog	86.9	n.r.	84.0	98.9	92.0	n.r.	n.r.	36.0

Hill et al., 2001	texturized soy protein	29	Adult dog	85.9	n.r.	83.1	98.9	91.3	n.r.	n.r.	34.0
Hill et al., 2001	texturized soy protein	57	Adult dog	83.9	n.r.	80.1	98.6	89.4	n.r.	n.r.	28.0
Hill et al., 2011	texturized soy protein	0	Dog	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	31
Hill et al., 2011	texturized soy protein	14	Dog	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	30
Hill et al., 2011	texturized soy protein	29	Dog	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	28
Hill et al., 2011	texturized soy protein	57	Dog	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	25
Hill et al., 2011	texturized soy protein	0	Dog	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	29
Hill et al., 2011	texturized soy protein	14	Dog	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	21
Hill et al., 2011	texturized soy protein	29	Dog	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	19
Hill et al., 2011	texturized soy protein	57	Dog	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	16

Félix et al., 2012	Regular soybean meal	0	Adult dog	79.3	86.3	81.1	94.7	86.4	n.r.	n.r.	41.6
Félix et al., 2012	Regular soybean meal	15	Adult dog	81.7	86.1	84.5	92.6	87.1	n.r.	n.r.	35.5
Félix et al., 2012	Regular soybean meal	30	Adult dog	80.6	84	84.1	90.7	84.9	n.r.	n.r.	29.8
Félix et al., 2012	high-protein soybean meal	0	Adult dog	79.9	86.1	81.4	94.5	86.7	n.r.	n.r.	41.2
Félix et al., 2012	high-protein soybean meal	15	Adult dog	81.9	86.3	83.6	92.7	87.0	n.r.	n.r.	34.8
Félix et al., 2012	high-protein soybean meal	30	Adult dog	82.6	86.2	84.5	91.9	86.8	n.r.	n.r.	29.0
Félix et al., 2013a	Defatted soybean meal	30	Adult dog	75.6	n.r.	85.1	84.3	79.8	n.r.	n.r.	31.5
Félix et al., 2013a	Soybean meal	30	Adult dog	75.8	n.r.	85.2	84.3	79.7	n.r.	n.r.	31.1
Félix et al., 2013a	Micronized soybeans	30	Adult dog	85.1	n.r.	88.4	96.8	88.8	n.r.	n.r.	31.5
Félix et al., 2013a	Toasted soybeans	30	Adult dog	76.7	n.r.	84.7	96.6	81.7	n.r.	n.r.	31.5

Félix et al., 2013a	Raw soybeans	30	Adult dog	75.9	n.r.	78.9	96.4	79.6	n.r.	n.r.	31.9
Félix et al., 2013a	Defatted soybean meal	30	Puppy	78.3	n.r.	84.8	93.9	81.3	n.r.	n.r.	28.2
Félix et al., 2013a	Soybean meal	30	Puppy	77.3	n.r.	85.2	95.8	80.1	n.r.	n.r.	28.7
Félix et al., 2013a	Micronized soybeans	30	Puppy	85.0	n.r.	87.4	98.2	87.8	n.r.	n.r.	29.4
Félix et al., 2013a	Toasted soybeans	30	Puppy	78.4	n.r.	84.5	98.5	82.4	n.r.	n.r.	28.3
Félix et al., 2013a	Raw soybeans	30	Puppy	75.6	n.r.	76.4	99.0	78.6	n.r.	n.r.	28.7
Tortola et al., 2013 exp1	Soybean meal	30	Adult dog	84.5	86.9	87.0	91.3	87.7	59.5	n.r.	30.7
Tortola et al., 2013 exp1	Soybean meal (after extrusion and drying - 7500 U protease/kg and 45 U cellulase/kg)	30	Adult dog	83.6	85.8	86.4	91.8	86.9	57.2	n.r.	32.1
Tortola et al., 2013 exp1	Soybean meal (after extrusion and drying - 15,000 U	30	Adult dog	83.7	86.4	85.8	91.9	87.1	60.8	n.r.	28.5

	protease/kg and 90 U cellulase/kg)										
Tortola et al., 2013 exp1	Poultry meal	28.9	Adult dog	85.6	87.6	85.9	91.7	88.1	63.0	n.r.	37.0
Tortola et al., 2013 exp2	Soybean meal	30	Adult dog	79.8	83.9	80.5	91.6	83.4	49.6	n.r.	30.6
Tortola et al., 2013 exp2	Soybean meal (after extrusion and drying - 140 U protease/kg; 8 U cellulase/kg, 800 U pectinase/kg, 60 U phytase/kg, 40 U betaglucanase/kg, 20 U xylanase/kg)	30	Adult dog	80.9	84.5	81.4	93.6	84.1	49.9	n.r.	29.2
Tortola et al., 2013 exp2	Soybean meal (after extrusion and drying - 700 U protease/kg, 40 U cellulase/kg, 4000	30	Adult dog	80.0	84.1	81.6	93.2	83.6	47.3	n.r.	31.6

	U pectinase/kg, 300 U phytase/kg, 200 U betaglucanase/kg and 100 U xylanase/kg)										
Tortola et al., 2013 exp2	Poultry meal	28.9	Adult dog	79.1	84.8	79.8	92.8	84.0	55.2	n.r.	41.7
Félix et al., 2013b	Soybean meal	30	Adult dog	85.2	84.7	89.8	86.6	87.2	n.r.	n.r.	31.5
Félix et al., 2013b	SPC600, 600g crude protein/kg	30	Adult dog	76.5	78.6	83.9	84.5	80.0	n.r.	n.r.	29.8
Félix et al., 2013b	SPC700, 700g crude protein/kg	30	Adult dog	77.2	78.4	85.2	85.4	83.9	n.r.	n.r.	42.2
Félix et al., 2013b	HSPC700, hydrolysed soy protein concentrate with 700g crude protein/kg	30	Adult dog	86.2	85.5	90.6	87.9	84.9	n.r.	n.r.	30.9
Félix et al., 2013b	SPI, soy protein isolate	30	Adult dog	91.6	92.5	98.8	81.7	93.4	n.r.	n.r.	31.4

Menniti et al., 2014	Soybean meal	0	Adult dog	81.1	85.0	81.3	91.2	n.r.	n.r.	n.r.	32.7
Menniti et al., 2014	Soybean meal	6	Adult dog	80.2	83.9	80.9	91.0	n.r.	n.r.	n.r.	32.4
Menniti et al., 2014	Soybean meal	11.5	Adult dog	80.9	84.5	82.1	91.8	n.r.	n.r.	n.r.	30.8
Menniti et al., 2014	Soybean meal	17	Adult dog	81.4	85.0	83.1	92.0	n.r.	n.r.	n.r.	30.2
Marx et al., 2015	soybean oil	0	Adult dog	68.7	77.7	80.8	86.3	77.4	n.r.	n.r.	34.7
Marx et al., 2015	soybean oil	6.5	Adult dog	70.4	78.9	80.8	78.9	80.2	n.r.	n.r.	35.1
Marx et al., 2015	soybean oil	13	Adult dog	73.4	81.1	81.4	79.8	83.1	n.r.	n.r.	35.8
Beloshapka et al., 2016	bioprocessed soy protein (HP300)	0	Adult dog	83.0	88.5	82.9	95.3	88.8	n.r.	n.r.	41.7
Beloshapka et al., 2016	bioprocessed soy protein (HP300)	4	Adult dog	84.6	89.4	85.8	94.4	89.6	n.r.	n.r.	39.4
Beloshapka et al., 2016	bioprocessed soy protein (HP300)	8	Adult dog	84.7	89.2	86.2	95.5	89.6	n.r.	n.r.	34.7

Beloshapka et al., 2016	bioprocessed soy protein (HP300)	12	Adult dog	82.2	87.4	84.4	95.4	87.9	n.r.	n.r.	34.9
Beloshapka et al., 2016	bioprocessed soy protein (HP300)	24	Adult dog	81.2	86.2	84.5	95.0	87.2	n.r.	n.r.	27.0
Beloshapka et al., 2016	bioprocessed soy protein (HP300)	48	Adult dog	77.5	82.6	86.0	93.4	84.0	n.r.	n.r.	28.6
Dhaliwal et al., 2016	soybean meal	34.9	Adult dog	85.5	78.6	82.8	69.1	n.r.	n.r.	34.8	n.r.
Venturini et al., 2018	cSPC, coarse particle size	45	Adult dog	81.6	84.6	86.9	91.4	84.5	n.r.	n.r.	35.1
Venturini et al., 2018	sSPC, small particle size	45	Adult dog	82.2	85.6	87.5	92.6	84.3	n.r.	n.r.	41.0
Venturini et al., 2018	Poultry by-product meal	30.9	Adult dog	82.5	86.1	87.8	90.7	83.8	n.r.	n.r.	41.8
Venturini et al., 2018	Corn gluten meal	18.7	Adult dog	83.3	86.6	88.8	92.1	84.3	n.r.	n.r.	41.3
Félix et al., 2020	Raw soybeans	0	Adult dog	82.3	85.6	83.2	89.1	85.8	n.r.	n.r.	36.4
Félix et al., 2020	Raw soybeans	6	Adult dog	81.9	85.6	82.1	90.2	86.4	n.r.	n.r.	36.3

Félix et al., 2020	Raw soybeans	12	Adult dog	81.7	85.5	81.8	90.3	86.6	n.r.	n.r.	36.5
Félix et al., 2020	Raw soybeans	18	Adult dog	81.4	85.2	81.3	90.3	86.7	n.r.	n.r.	34.8
Félix et al., 2020	Raw soybeans	24	Adult dog	81.1	85.0	80.8	90.5	87.0	n.r.	n.r.	33.6
Félix et al., 2020	Raw soybeans	30	Adult dog	80.8	84.1	80.6	91.0	87.1	n.r.	n.r.	31.3
Pattanaik and Kore, 2021	Soybean meal (twice daily)	30	Adult dog	86.1	99.5	94.9	97.1	n.r.	n.r.	43.9	27.4
Pattanaik and Kore, 2021	Soybean meal (once daily)	30	Adult dog	86.1	87.8	95.2	97.3	n.r.	n.r.	43.0	25.2
Mohneet et al., 2021*	Soy nugget	0	N/A	92.2	91.3	92.8	94.9	n.r.	n.r.	n.r.	n.r.
Mohneet et al., 2021*	Soy nugget	5	N/A	88.2	87.9	88.1	88.9	n.r.	n.r.	n.r.	n.r.
Mohneet et al., 2021*	Soy nugget	10	N/A	89.3	89.9	89.7	91.8	n.r.	n.r.	n.r.	n.r.
Mohneet et al., 2021*	Soy nugget	15	N/A	88.4	88.3	89.0	90.2	n.r.	n.r.	n.r.	n.r.

1DM = dry matter, 2OM = organic matter, 3CP = crude protein, 4AHEE = acid hydrolyzed ether extract, 5GE = gross energy, 6TDF = total dietary fiber, 7CF = crude fiber.

*in vitro digestibility values for extruded dog feed

Table 1.5 Ileal total tract digestibility (small intestine digestibility or prececal digestibility) in companion animals fed soy included diets

Authors	Soy ingredients	Inclusion, %	Animal	Apparent total tract digestibility, %						
				[1]DM	[2]OM	[3]CP	[4]AHEE	[5]GE	[6]TDF	[7]CF
Yamka et al., 2003	Soybean meal	15	Adult dog	80.7	n.r.	65.4	n.r.	n.r.	n.r.	n.r.
Yamka et al., 2003	Soybean meal	26	Adult dog	71.0	n.r.	66.2	n.r.	n.r.	n.r.	n.r.
Yamka et al., 2003	Soybean meal	36	Adult dog	53.4	n.r.	59.8	n.r.	n.r.	n.r.	n.r.
Yamka et al., 2003	Soybean meal	46	Adult dog	33.8	n.r.	51.1	n.r.	n.r.	n.r.	n.r.
Yamka et al., 2005a	low-oligosaccharide whole soya beans	40.12	Adult dog	78.0	n.r.	71.7	n.r.	n.r.	n.r.	n.r.
Yamka et al., 2005a	low-oligosaccharide low-phytate whole soya beans	45.21	Adult dog	76.4	n.r.	75.1	n.r.	n.r.	n.r.	n.r.
Yamka et al., 2005a	soya bean meal	31.73	Adult dog	80.8	n.r.	78.2	n.r.	n.r.	n.r.	n.r.
Yamka et al., 2005b	low-oligosaccharide, low-phytate soybean meal	29.22	Adult dog	80.9	n.r.	79.2	n.r.	n.r.	n.r.	n.r.
Yamka et al., 2005b	conventional soybean meal	30.85	Adult dog	77.5	n.r.	82.0	n.r.	n.r.	n.r.	n.r.
Yamka et al., 2005b	low-oligosaccharide, low-phytate whole soybean	45.25	Adult dog	74.0	n.r.	68.8	n.r.	n.r.	n.r.	n.r.
Yamka et al., 2005b	conventional whole soybean	40.1	Adult dog	76.1	n.r.	69.8	n.r.	n.r.	n.r.	n.r.
Hill et al., 2000	texturized soy protein	0	Adult dog	80.6	n.r.	77.0	99.4	n.r.	n.r.	n.r.
Hill et al., 2000	texturized soy protein	14	Adult dog	77.1	n.r.	73.4	99.5	n.r.	n.r.	n.r.

Hill et al., 2000	texturized soy protein	29	Adult dog	75.2	n.r.	71.8	98.8	n.r.	n.r.	n.r.
Hill et al., 2000	texturized soy protein	57	Adult dog	71.7	n.r.	70.8	99.4	n.r.	n.r.	n.r.
Hill et al., 2001	texturized soy protein	0	Adult dog	80.6	n.r.	77.0	99.4	89.1	n.r.	n.r.
Hill et al., 2001	texturized soy protein	14	Adult dog	77.1	n.r.	73.4	99.5	86.9	n.r.	n.r.
Hill et al., 2001	texturized soy protein	29	Adult dog	75.2	n.r.	71.8	98.8	85.5	n.r.	n.r.
Hill et al., 2001	texturized soy protein	57	Adult dog	71.7	n.r.	70.8	99.4	83.2	n.r.	n.r.

1DM = dry matter, 2OM = organic matter, 3CP = crude protein, 4AHEE = acid hydrolyzed ether extract, 5GE = gross energy, 6TDF = total dietary fiber, 7CF = crude fiber.

Table 1.6 Apparent total tract digestibility and fecal dry matter (%) in companion animals fed diets containing soybean hulls

Authors	Soy ingredients	Inclusion, %	Animal	Apparent total tract digestibility, %							Fecal DM, %
				[1]DM	[2]OM	[3]CP	[4]AHEE	[5]GE	[6]TDF	[7]CF	
Burkhalter et al., 2001	Soybean hulls (Central Soya)	7.5	Adult dog	72.7	79	74.1	92.6	81.2	-8.5	n.r.	36.0
Burkhalter et al., 2001	Soybean hulls (Cargill)	7.5	Adult dog	70.9	78	72.7	92	80.3	-9.6	n.r.	37.0
Burkhalter et al., 2001	Soybean hulls (Jones-A)	7.5	Adult dog	74.6	81.7	78.4	94	83.8	11.6	n.r.	36.0
Burkhalter et al., 2001	Soybean hulls (Quincy)	7.5	Adult dog	69.2	77	70.9	91.5	80.5	-7.3	n.r.	32.0
Burkhalter et al., 2001	Soybean hulls (Jones-B)	7.5	Adult dog	71.3	78.4	73.9	92.2	80.7	-10.4	n.r.	36.0
Sabchuk et al., 2017	Soy hull	0	Adult dog	84.0	n.r.	88.9	91.1	89.0	n.r.	n.r.	37.1
Sabchuk et al., 2017	Soy hull	4	Adult dog	80.1	n.r.	85.2	85.7	83.7	n.r.	n.r.	31.1
Sabchuk et al., 2017	Soy hull	8	Adult dog	78.8	n.r.	86.4	89.5	89.8	n.r.	n.r.	35.9
Sabchuk et al., 2017	Soy hull	12	Adult dog	73.8	n.r.	84.2	86.8	80.1	n.r.	n.r.	34.4
Sabchuk et al., 2017	Soy hull	16	Adult dog	71.9	n.r.	83.4	85.9	78.8	n.r.	n.r.	35.4
Detweiler et al., 2019a	Soybean hull	0	Adult dog	85.4	90.1	85.8	90.9	n.r.	37.8	n.r.	44.7
Detweiler et al., 2019a	Soybean hull	15	Adult dog	79.6	79.9	83.3	91.9	n.r.	22.7	n.r.	39.4

Detweiler et al., 2019b	Soybean hull	0	Adult cat	85.5	88.8	84.9	89.9	n.r.	8.5	n.r.	35.0
Detweiler et al., 2019b	Soybean hull	14	Adult cat	75.4	78.5	81.7	88.6	n.r.	18	n.r.	38.9

1DM = dry matter, 2OM = organic matter, 3CP = crude protein, 4AHEE = acid hydrolyzed ether extract, 5GE = gross energy, 6TDF = total dietary fiber, 7CF = crude fiber.

Table 1.7 Fresh fecal pH, ammonia, short-chain fatty acids, branched-fatty acids, lactate, phenol, and indole concentration presented in µmol/g DM fecal samples when fed soys (different units specified with footnotes).

Authors	Soy ingredients	Inclusion, %	Animal	Fecal pH	Short-chain fatty acid				Branched-chain fatty acid				Lactate	Ammonia	Phenol	Indole
					Acetate	Propionate	Butyrate	Total SCFA	Valerate	Isovalerate	Isobutyrate	Total BCFA				
Pawar and Pattanaik, 2009	soybean meal	50	dog	4.9	260.6	170.4	39.8	470.7	n.r.	n.r.	n.r.	n.r.	14.6	10.5	n.r.	n.r.
Pawar and Pattanaik, 2009	soya nugget	50	dog	5.1	121.8	65.3	27.4	214.6	n.r.	n.r.	n.r.	n.r.	7.9	6.1	n.r.	n.r.
Félix et al., 2012	soybean meal	15	dog	6.6	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Félix et al., 2012	soybean meal	30	dog	6.3	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Félix et al., 2012	high-protein	15	dog	6.7	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.

Félix et al., 2012	soybean meal high-protein soybean meal	30	dog	6.5	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Félix et al., 2013a	Defatted soybean meal	30	dog	5.9	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	230.2	n.r.	n.r.
Félix et al., 2013a	Soybean meal	30	dog	5.8	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	220.2	n.r.	n.r.
Félix et al., 2013a	Micronized soybeans	30	dog	5.9	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	219.6	n.r.	n.r.
Félix et al., 2013a	Toasted soybeans	30	dog	6.9	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	227.8	n.r.	n.r.
Félix et al., 2013a	Raw soybeans	30	dog	6.9	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	259.0	n.r.	n.r.
Félix et al., 2013a	Defatted soybean meal	30	puppy	5.6	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	307.1	n.r.	n.r.

Félix et al., 2013a	Soybean meal	30	puppy	5.6	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	299.5	n.r.	n.r.
Félix et al., 2013a	Micronized soybeans	30	puppy	5.6	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	303.0	n.r.	n.r.
Félix et al., 2013a	Toasted soybeans	30	puppy	6.6	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	296.5	n.r.	n.r.
Félix et al., 2013a	Raw soybeans	30	puppy	6.6	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	330.0	n.r.	n.r.
Tortola et al., 2013 exp1	Soybean meal	30	dog	6.2	272	244	22	539	n.r.	n.r.	n.r.	n.r.	16.7	76.3	n.r.	n.r.
Tortola et al., 2013 exp1	Soybean meal[a]	30	dog	6.3	246	208	33	488	n.r.	n.r.	n.r.	n.r.	17.0	70.5	n.r.	n.r.
Tortola et al., 2013 exp1	Soybean meal[b]	30	dog	6.1	246	196	27	470	n.r.	n.r.	n.r.	n.r.	18.8	88.1	n.r.	n.r.
Tortola et al., 2013 exp2	Soybean meal	30	dog	5.9	324	245	56.8	324	n.r.	n.r.	n.r.	n.r.	20.1	70.5	n.r.	n.r.

Tortola et al., 2013 exp2	Soybean meal[c]	30	dog	5.9	356	313	41.9	356	n.r.	n.r.	n.r.	n.r.	29.2	70.5	n.r.	n.r.
Tortola et al., 2013 exp2	Soybean meal[d]	30	dog	6.0	370	306	43.1	370	n.r.	n.r.	n.r.	n.r.	16.8	76.3	n.r.	n.r.
Félix et al., 2013b	soybean meal	30	dog	6.5	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	146.2*	n.r.	n.r.
Félix et al., 2013b	SPC600	30	dog	6.6	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	158.0*	n.r.	n.r.
Félix et al., 2013b	SPC700	30	dog	6.4	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	160.3*	n.r.	n.r.
Félix et al., 2013b	HSPC700 (hydrolysed SPC)	30	dog	6.5	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	157.4*	n.r.	n.r.
Félix et al., 2013b	SPI	30	dog	7.0	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	153.3*	n.r.	n.r.

Beloshapka et al., 2016	biopressed soy protein (HP300)	4	dog	6.6	162.0	93.4	39.2	294.6	0.7	12.4	7.8	20.9	n.r.	117.7	2.2	2.2
Beloshapka et al., 2016	biopressed soy protein (HP300)	8	dog	6.4	180.8	96.0	32.3	309.1	0.6	9.0	5.8	15.3	n.r.	116.6	1.2	2.2
Beloshapka et al., 2016	biopressed soy protein (HP300)	12	dog	6.2	224.2	109.8	46.3	380.3	0.7	9.0	6.0	15.7	n.r.	114.7	1.1	1.8
Beloshapka et al., 2016	biopressed soy protein (HP300)	24	dog	6.3	367.7	177.8	48.3	593.7	1.3	8.5	5.9	15.7	n.r.	134.2	0.7	0.7
Beloshapka et al., 2016	biopressed soy protein (HP300)	48	dog	6.4	318.0	188.8	40.0	546.7	1.2	5.1	3.5	9.9	n.r.	70.5	0.3	0.8

Venturini et al., 2018	cSPC, coarse particle size	45	dog	6.2	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	4.1	94	n.r.	n.r.
Venturini et al., 2018	sSPC, small particle size	45	dog	6.4	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	102	n.r.	n.r.
Félix et al., 2020	Raw soybeans	6	dog	6.4	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Félix et al., 2020	Raw soybeans	12	dog	6.5	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Félix et al., 2020	Raw soybeans	18	dog	6.5	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Félix et al., 2020	Raw soybeans	24	dog	6.6	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Félix et al., 2020	Raw soybeans	30	dog	6.6	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Pattanaik and Kore, 2021	Soybean meal	30	dog	5.2	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.

Pattanaik and Kore, 2021	(twice daily) Soybean meal (once daily)	30	dog	5.4	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	4.5	n.r.	n.r.	n.r.

Soybean meal[a] (after extrusion and drying 7500 U protease/kg and 45 U cellulase/kg)

Soybean meal[b] (after extrusion and drying 15 000 U protease/kg and 90 U cellulase/kg)

Soybean meal[c] (after extrusion and drying 140 U protease/kg; 8 U cellulase/kg, 800 U pectinase/kg, 60 U phytase/kg, 40 U betaglucanase/kg, 20 U xylanase/kg)

Soybean meal[d] (after extrusion and drying 700 U protease/kg, 40 U cellulase/kg, 4000 U pectinase/kg, 300 U phytase/kg, 200 U betaglucanase/kg and 100 U xylanase/kg)

*Ammonia concentration μmol/g as-is fecal sample

Table 1.8 Fresh fecal pH, ammonia, short-chain fatty acids, branched-fatty acids, lactate, phenol, indole, and skatole concentration presented in µmol/g DM fecal samples when fed soy hulls otherwise specified with footnotes.

Authors	Soy ingredients	Inclusion, %	Animal	Fecal pH	Short-chain fatty acid				Branched-chain fatty acid				Lactate	Ammonia	Phenol	Indole	Skatole
					Acetate	Propionate	Butyrate	Total SCFA	Valerate	Isovalerate	Isobutyrate	Total BCFA					
Sabchuk et al., 2017	Soy hull	4	dog	5.9										34.6*			
Sabchuk et al., 2017	Soy hull	8	dog	6.8										59.9*			
Sabchuk et al., 2017	Soy hull	12	dog	6.6										46.4*			
Sabchuk et al., 2017	Soy hull	16	dog	6.6										54.6*			
Myint et al., 2017	Soybean husk	5.6	dog	5.9	91.2	54.5	10.6	158.3						0.1		4.1	3.8

Detweiler et al., 2019	Soy hull	15	dog	5.9	321.0	121.0	37.7	479.7	0.9	9.8	6.3	17	147.8	0.3	1.4
Detweiler et al., 2019	soybean hull	14	cat	5.7	274.3	76.2	72.1	422.7	5.8	13.4	7.6	26.8	130.5	0.5	1.4

Soybean meal[a] (after extrusion and drying 7500 U protease/kg and 45 U cellulase/kg)

Soybean meal[b] (after extrusion and drying 15 000 U protease/kg and 90 U cellulase/kg)

Soybean meal[c] (after extrusion and drying 140 U protease/kg; 8 U cellulase/kg, 800 U pectinase/kg, 60 U phytase/kg, 40 U betaglucanase/kg, 20 U xylanase/kg)

Soybean meal[d] (after extrusion and drying 700 U protease/kg, 40 U cellulase/kg, 4000 U pectinase/kg, 300 U phytase/kg, 200 U betaglucanase/kg and 100 U xylanase/kg)

*Ammonia concentration μmol/g as-is fecal sample

Chapter 2 – Internal versus external fat in extrusion of dry expanded dog kibbles containing soy – Impact on process stability and product uniformity

2.1 Abstract

Pet food does not exceed 20% fat due to lubrication and related processing and product quality issues; however, increased fat is needed for premium pet foods. Use of whole soybeans (WSB) containing internal fat may alleviate this problem. The objective of this study was to determine the effect of WSB on process stability and product physicochemical characteristics during extrusion of dry dog food.

Using a 2 × 3 factorial arrangement of treatments with 2 levels of fat (high fat; HF vs. low fat; LF) and 3 fat insertion sites (no fat; NO vs. internal fat; IN, from WSB vs. external fat; EX, from soybean oil; SBO), six dog diets were produced by a pilot-scale single screw extruder. Extruder screw speed was adjusted to maintain wet product bulk density at ~350 g/L. Physical properties and physicochemical characteristics were analyzed. Data were statistically analyzed using a GLM procedure for mixed models in statistical software (GLIMMIX, SAS version 9.4) with fat content and fat insertion site as fixed effects. Results were considered significant at $P < 0.05$.

The EX required the highest extruder screw speed (average; 404 rpm), to achieve the target bulk density, followed by IN and NO (351 and 309 rpm, respectively). The EX led to process instability including extruder surging, and higher variability in kibble dimensions as indicated by the calculated variance. The EX had higher sectional expansion index (SEI) and lower specific length (SL; $P < 0.05$), leading to similar bulk density to IN and CO. Peak force and compression

area for LF were higher ($P < 0.05$) than those for HF. The HF had lower ($P < 0.05$) peak viscosity, breakdown, hold viscosity, final viscosity, and setback compared to the LF treatments due to the lower starch content. The total processing energy was greater for LF than HF due to the higher steam retention. There was heat loss from the material to the barrel in the extruder for all treatments due to material temperature being higher than barrel temperature.

The SBO adversely affected extruder stability and product expansion compared to WSB. By utilizing WSB in the formula in exchange for liquid fat, the pet food industry might be able to increase the energy density and palatability of kibbles, while keeping cost of processing low. Having $42 billion value in 2021 of the pet food market, these findings have the potential for substantial economic impact.

2.2 Introduction

Soybeans are a quality protein source. However, they have an undeserved reputation as a poor-quality ingredient in modern pet food. Some claim that soy may lead to allergies, is a cheap ingredient, a filler, a low nutritional quality ingredient. Soybean contains anti-nutritional compounds, including trypsin inhibitors, oligosaccharides, and phytate phosphorus which decrease nutrient utilization in monogastric animals (Kim and Aldrich, 2023). However, the heat process during extrusion can alter the structure of these proteins and render them inactive (Riaz, 2000).

The pet products industry has reached $ 103.6 billion in annual sales having $ 42 billion was spent on pet food and treats in 2021 (APPA, 2021). With this market growth, one of new requests from pet food purchasers is a high energy density dry dog food with high fat contents. However, the pet food industry is limited in the amount of fat that can be added to kibble due to processing challenges. There is a restriction on the amount of fat that can be coated outside of the kibbles without a vacuum coater. Introducing the liquid fat to the ration during extrusion may

negatively affect product expansion and bulk density due to fat lubrication. As fat levels increase in the barrel, it is difficult to transmit mechanical energy from the screws into the product as fat reduces friction within the extruder barrel (Rokey, 2006). Fat is also reported to coat feed particles preventing moisture absorption and thermal heat transfer required for starch gelatinization during extrusion (Rokey, 2006). It was hypothesized that ingredients like whole soybeans (WSB), internal fat source, may increase energy density while avoiding production and product quality issues compared to the external fat source, soybean oil (SBO).

A preliminary study conducted in our lab investigated the effect of whole soybeans (WSB) on extrusion and product parameters of dry dog food (Kim and Aldrich, 2023). Experimental diets were produced in a single screw extruder at 0, 100, 200, and 300 g/kg, as fed WSB inclusion. The input production parameters were common across treatments to determine the effect of WSB on output parameters. The increased inclusion level of WSB changed processing conditions and outputs by the lubrication effect from internal fat contents. Based on this preliminary work, a second study was conducted with a laboratory-scale co-rotating twin-screw extruder (Micro-18, American Leistritz, Somerville, NJ, USA) to further evaluate the effect of internal (addition of full-fat soy) vs external fat (addition of SBO) inclusion on extrusion processing. For this study, three treatments were formulated: no fat inclusion (NOF; pet food mix + soybean meal; SBM), internal fat inclusion (INF; pet food mix + 200 g/kg as fed full-fat soy), and external fat inclusion (EXF; pet food mix + SBM + SBO). The INF and EXF had decreased die temperature and motor load compared to NOF. In addition, INF treatment had decreased die pressure compared to the NOF. Overall, the addition of full-fat soy and SBO decreased the expansion of the products when compared to NOF. Increasing feed rate and barrel temperature increased expansion for INF treatment. However, EXF treatment never produced an ideal product, even with adjustments in

processing conditions. From this work, there appeared to be benefit from internal fat provided via the internalized fat of an ingredient compared to liquid external addition. The full-fat soy led to a product more similar compared to the NOF.

Additionally, it was learned in the lab-scale extruder study that the introduction of external fat into the extruder created an obstacle to stable product production. It was hypothesized that the EXF treatment might be stabilized with a preconditioning step and increased energy input from a larger scale extruder. The preconditioning is known to assist starch cook and aid expansion of products (Rokey, 2006). It was also hypothesized that the internal fat from whole soybeans would resulting in less lubrication impact on extrusion conditions (motor load, motor power, die temperature, and specific mechanical energy; SME) and the final product qualities (bulk density, product expansion and variability of kibbles dimensions) than the external fat inclusion. In other words, the whole soybean-containing diet would require higher energy input to reach the required SME to cook the product than the EXF when targeting the same extrudate bulk density. Manipulating processing input conditions might respect in the same product quality as the control diet when producing the high-fat formulas. Therefore, the objective of this project was to determine the effect of WSB with internal fat on the product quality (i.e., bulk density) using a pilot-scale single screw extruder.

2.3 Materials and methods

2.3.1 Experimental design and diets

The experiment was designed as a 2 × 3 factorial arrangement of treatments, with two levels of fat (high fat content; HF, and low fat content; LF) and three fat insertion sites (negative control with no additional fat; NO, internal fat derived from whole soybean; IN, and external fat derived from soybean oil; EX). A typical base formula (BASE) that met the Association of

American Feed Control Officials (AAFCO) for adult dog maintenance was used for production (Table 2.1). The six dietary treatments were: soybean meal; SBM (NOHF), 200 g/kg as fed whole soybeans (INHF), SBM + soybean oil; SBO (EXHF), SBM (NOLF), 150 g/kg as fed whole soybeans (INLF), SBM + SBO (EXLF). The two control treatments (NOHF and NOLF) were formulated to mimic traditional pet food processing.

Raw materials for the base ration were purchased from and blended by a commercial mill (Fairview Mills, Seneca, KS, USA), with particle size reduced via a hammer mill to pass through a 2-mm screen. WSB was purchased from a local grain elevator (MKC; Manhattan, KS, USA), cleaned, ground with a hammer mill (model 18-7-300; Schutte Buffalo, NY, USA) to pass through a 1.19-mm screen (3/64") at Hal Ross Mill (Manhattan, KS, USA). Experimental treatments were mixed in 136 kg batches using a double ribbon mixer for 5 min prior to extrusion.

2.3.2 Extrusion processing

The pet food products were produced using a single screw extruder (X-20, 37.3 kW; Wenger Manufacturing, Sabetha, KS, USA). The feeder screw was calibrated in duplicates for the first set of treatments to calculate feed rate prior to extrusion. Products with similar nutrient compositions were expected to have similar feed rates; therefore, the same feed rates from HF treatments were used for the LF treatments. The screw profile (Figure 1) consisted of a diameter 3.25 inch (82.55 mm), one tapered circular die with a 25-mm inlet diameter, 6.22-mm die opening diameter, 18.6 mm thickness, and 2.75 mm land length was used along with a six hard knife arrangement rotating at 1106 rpm.

The NOHF was produced first to determine the standard bulk density and set the visual standard for an idealized pet food product. The target bulk density of 350 g/L was established to mimic traditional pet food products (280-400 g/L; Rokey, 2006). Bulk density (BD) was

measured by filling a cylindrical steel container (V_c = 1 L) with extrudates and recording the mass for calculation using Equation 1.

$$BD, \frac{g}{l} = W_{sample}/V_c \quad (1)$$

where, W_{sample} is the mass of extrudates (g), and V_c is the volume of the steel cylinder.

After establishing the production conditions for the NOHF, EXHF and EXLF were produced using the same processing conditions as the control. After measuring the bulk density, extruder screw speed was adjusted to achieve the same bulk density to the NOHF. For the LF treatments, the production was done in the same manner; NOLF, EXLF, and then INLF. The standard bulk density and processing conditions for the LF treatments were determined from NOLF. The BASE was used to start up the extruder and to lower the die temperature to about 80 °C for shutdown.

During processing, feed rate, water, and steam inclusion in the preconditioner and into the extruder, temperature off the preconditioner and at the extruder die, extruder screw speed, die pressure, extruder motor load, extruder motor power, and mass flow rate were recorded (Table 2.2). The mass flow rate was measured in duplicates by collecting the extrudates for 1 minute off the extruder. Using the recorded processing parameters, specific mechanical energy (SME), and in-barrel moisture (MC) were calculated. The SME was determined by Equation 2 (Webb et al., 2020).

$$SME\ (\frac{kJ}{kg}) = \frac{\text{operational kW of extruder} - \text{no load kW of extruder}}{\text{dry feed rate (kg/s)}} \quad (2)$$

In-barrel moisture content (MC) was determined by Equation 3.

$$MC = \frac{m_f \times X_f + m_{ps} + m_{pw} + m_{es} + m_{ew}}{m_f + m_{ps} + m_{pw} + m_{es} + m_{ew}} \quad (3)$$

where, m_f is the dry feed rate (≅ feeder screw speed x 10 kg/h for KSU single screw with volumetric feed system); X_f is the wet basis moisture content of the feed material (estimated at 10 %); m_{ps} is the steam injection rate in the pre-conditioner (kg/h); m_{pw} is water injection rate in the pre-conditioner (kg/h); m_{es} is the steam injection rate in the extruder (kg/h); and m_{ew} is water injection rate in the extruder (kg/h). Additionally, wet samples off the preconditioner, extrudate samples off the extruder, and dried samples off the drier were collected in duplicates for each treatment. The collected samples were stored in at -20 °C for further analysis.

2.3.3 Physical characteristics analysis

The length and diameter of dried kibbles were determined by measuring 10 pieces in 3 replicates (collected at various times from the processing stream) per treatment using 6" digital calipers. The kibble dimension variability was determined by calculating the standard deviation of the treatments. The mass of dried kibbles was also measured in duplicate in 3 replicates per treatment. Sectional expansion ratio/index (SEI) was determined by comparing the squared diameter of the dried extruded kibbles by the squared die diameter of the extruder, Equation 4 (Manepalli et al., 2019).

$$Sectional\ expansion\ ratio/index = \frac{(D)\text{^}2}{(d)\text{^}2} \quad (4)$$

where, D is the extrudate diameter and d is extruder die diameter.

Specific length in cm/g was determined by dividing the length of the extrudate by its mass, Equation 5 (Shukri et al., 2021).

$$Specific\ length, cm/g = \frac{l}{m} \quad (5)$$

where, l is the extrudate length and m is extrudate mass.

The moisture content was measured following the American Association for Cereal Chemistry method 44-19.01 (AACC, 1999). Approximately 1.5 g of each ground sample was dried at 135 °C for 2 h in duplicates.

Compression test was performed with a Texture Analyzer (TA.XT2i; Texture Technologies Corp., Scarsdale, NY, USA), equipped with a 25 mm cylindrical probe. Samples were compressed with a test speed of 2 mm/sec and 50% strain along the direction of extrusion (length of the kibble). Peak force (kg) and compression test area (kg*sec) was recorded for 15 randomly selected dried kibbles per treatment by a computer software (Exponent version 5).

The pasting properties of the treatments were analyzed based on the method of Shukri and Shi (2017) using a rapid visco analyzer (RVA4500, Perten Instruments, IL, USA). For analysis, 3.5 g of finely ground samples in duplicates were suspended in 25.0 g distilled water. The heating and cooling cycles were programmed by heating the suspension from 50 °C to 90 °C (heating rate of 5 °C/min), held at 90 °C for 6 min, and cooled to 50 °C (cooling rate of 5 °C/min). Parameters collected included peak viscosity, breakdown viscosity, holding viscosity, setback viscosity, and final viscosity. Peak time and pasting temperature were also obtained. Each sample was analyzed in duplicate.

2.3.4 Mass and energy balance

Mass and energy balance analyses were conducted based on the description provided by Maichel (2021). Steam loss from the preconditioner was calculated using mass balance principles; wherein, total mass entering the preconditioner equals the total mass exiting the preconditioner. Therefore, the mass of the steam lost from the preconditioner or not absorbed in the preconditioner (m_{slpc}) equals the sum of the mass of the input streams minus the mass of the preconditioned material exiting the preconditioner, as follows.

$$m_{slpc} = \left(m_r + m_{spc} + m_{wpc}\right) - m_{pc} \quad (6)$$

where m_{spc} is the mass flow rate of the steam entering the preconditioner (kg/h), m_r is the flow rate of ration into the preconditioner, m_{spc} is the flow rate of steam injected into the preconditoner, m_{wpc} is the mass flow rate of the water entering the preconditioner (kg/h) and m_{pc} is the mass of the preconditioned material exiting the preconditioner (kg/h).

The flow rates m_r, m_{spc} and m_{wpc} were known input variables. The mass of the material exiting the preconditioner, m_{pc}, was calculated from mass balance of solids using the following equation:

$$m_{pc} = m_r\left(\frac{1-x_{wr}}{1-x_{wpc}}\right) \quad (7)$$

where x_{wr} is the moisture content of the raw material (% wet basis) and x_{wpc} is the content of material leaving the preconditioner (% wet basis).

Specific Thermal Energy (STE) was calculated based on the energy of steam absorbed into raw material in the preconditioner as follows:

$$STE = Q_{steam}/m_r \quad (8)$$

where Q_{steam} is the energy of the steam absorbed in the preconditioner. This is the energy of the steam injected minus the energy lost through the steam loss, and is calculated as follows:

$$Q_{steam} = Q_{spc} - Q_{slpc} \quad (9)$$

where Q_{spc} are Q_{slpc} are the energy of steam (kJ/h) injected into the preconditioner and lost from the preconditioner (kg/h), respectively, and are calculated from steam enthalpies and mass flow rates as shown below.

$$Q_{spc} = h_{spc} \times m_{spc} \quad (10)$$

$$Q_{slpc} = h_{slpc} \times m_{slpc} \quad (11)$$

where h_{spc} and h_{slpc} are the enthapies of steam (kJ/kg) injected into the preconditioner and lost from the preconditioner, respectively, as obtained from steam tables based on the steam pressure.

Similarly, energy balance principles were used to calculate energy input from extruder barrel to the material.

2.3.5 Statistical analysis

Physical characteristics were statistically analyzed using a GLIMMIX procedure with SAS (2013) with fat content (HF vs. LF) and fat insertion site (NO vs. IN vs. EX) as fixed effects. Results were considered significant at $P < 0.05$ and the superscripts were determined based on the interaction in result tables.

2.4 Results and Discussion

2.4.1 Experimental design and diets

Final products were formulated to contain the same crude protein and crude fat content for each set of treatments after coating. However, the calculated nutritional composition for the diet rations that went into the extruder is presented in Table 2.1. To minimize this change due to adjustment of ingredients, corn was added to the LF treatments to match the HF treatments. The net results were that the addition of corn, led to nitrogen free extract (NFE) of LF treatments being greater than HF treatments. Furthermore, the addition of WSB increased the fiber content of both INHF and INLF compared to the other treatments. The unintentional change in NFE and fiber content may impact processing parameters and physical properties of the final products in addition to fat inclusion.

2.4.2 Extrusion processing

The extruder shaft speed for experimental treatments was adjusted to achieve the same bulk density for the off-extruder kibbles based on each negative control (NOHF and NOLF) (Table 2.2). The high extruder shaft speed for EXHF indicated that it required higher mechanical energy input than NOHF to produce well expanded products similar to NOHF. It is known that higher screw speeds will increase mechanical energy and decrease bulk density during extrusion (Rokey, 2006). The extruder screw speed required to produce INHF to meet the target bulk density of 350 g/L was higher (352 rpm) than that for NOHF (305 rpm) but lower than EXHF (406 rpm). This result validated the hypothesis that internal fat inclusion derived from an ingredient does have less negative effects on product expansion than external fat inclusion. Other input variables remained the same.

Similar to the production of HF treatments, the determination of processing conditions was determined from production of ideal bulk density for NOLF. Processing conditions for NOLF were similar to that of NOHF. This was expected as the base formulas only varied slightly with an increase in corn inclusion. Shaft speed adjustments for EXLF and INLF followed the same trend as EXHF and INHF to reach similar bulk densities; except for the extruder shaft speed, all the other processing conditions remained unchanged.

When compared to negative controls (NOHF and NOLF), the production of experimental treatments resulted in a decreased, and fluctuating percent motor load due to the addition of fat (Table 2.2). Fat has been reported to cause materials to slip within the barrel and lower the friction, decrease extruder motor load, and result in poor product expansion due to insufficient pressure during extrusion (Riaz, 2000). Moreover, the inconsistent results for motor load likely indicates surging of the extruder due to fat inclusion into the barrel (Luker and Cedar Grove, 1996).

However, the motor power and die temperature increased due to the higher extruder shaft speed (Table 2.2). The SME can also be affected by the higher extruder shaft speed (Rokey et al, 2010). It was predicted that the addition of fat would lower SME. But, to increase cook and improve final product quality, the screw speed was increased which may have influenced the results. In addition, in-barrel moisture content was unintentionally decreased during production of the EXHF and INHF. This could have also led to the increase in SME (Kantrong, 2018). The SME for the negative controls (NOHF and NOLF) and WSB treatments (INHF and INLF) were similar with increased extruder shaft speed, meaning that the internal fat derived from WSB did not act as a lubricant within the barrel.

2.4.3 Physical characteristics

There was no difference in off-drier bulk density for the LF treatments while EXHF was higher than INHF ($P < 0.05$) (Table 2.3). The off-extruder bulk density for the IN treatments were higher than the controls, NO treatments ($P < 0.05$) (Table 2.4). Rokey et al., (2010) reported that a 1% increase in fat between 12-17% of the formula will increase the bulk density of the product by 16 g/L. In this experiment, the off-drier bulk density for LF treatments was maintained in a narrow range (334.0 – 340.0 g/L) indicating that processing conditions were accurately adjusted during production when the fat content was low. For HF treatments, however, the off-drier bulk density was impacted differently with the adjustment by fat inclusion insertion site into the processing.

The dried kibble diameter and SEI for EX treatments were higher (average; 11.5 mm and 3.4, respectively; $P < 0.05$) than the NO treatments (average; 11.0 mm and 3.1, respectively) which was likely attributed to the higher SME within the barrel. On the other hand, there was no difference in the dried kibble diameter and SEI between IN treatments and the NO treatments (P

> 0.05). The dried kibble length for IN treatments (average; 6.4 mm) were lower than the other treatments (average; 6.7 mm; $P < 0.05$). The fiber content in the WSB was 200 g/kg as fed total dietary fiber on a dry matter basis (Kim and Aldrich, 2023), which might have affected the kibbles resulting in less expansion. According to the Guy classification system, fibers are dispersed phase fillers and known to have very poor functionality in extrusion (Guy, 2001). High fiber ingredients are also associated with low expansion of extrudates (Pai et al. 2009).

Interestingly, the dried kibble for EX treatments were heavier (average; 0.38 g) and had lower specific length (average; 1.8 cm/g) than the other treatments (average; 0.31 g and 2.1 cm/g, respectively; $P < 0.05$). This result indicates that the external fat decreased product expansion horizontally. It has been reported that fat addition has a negative effect on product expansion during extrusion (Rokey et al., 2010). On the other hand, IN treatments had no difference in specific length to the NO treatments. Rokey et al., (2010) reported that endogenous fats, supplied as a component of an ingredient, typically have less effect on expansion than refined fats. In addition, the same specific length and SEI for IN treatments and NO treatments could indicate that processing parameters were adjusted appropriately to promote product expansion with the internal fat inclusion.

Kibble dimensions (diameter and length) were more variable within EX treatments than IN treatments as indicated by the higher coefficient of variation (Figure 2). The increase in variability is likely due to surging which was observed during production. Khan et al. (2014) stated that extruder surging results in product thickness variation. However, there was less variability in kibble dimensions for the EXLF treatment compared to the EXHF. This indicates that the decrease in fat content resulted in less surging and more uniform kibble. Even with the increase in variability

between EX treatments, the standard deviation for kibble length and width were low (less than 1.30) for all treatments.

Peak force and compression area for LF treatments were higher (average; 17.66 kg and 16.18 kg*sec, respectively) than those for HF treatments (average; 15.4 kg and 13.87 kg*sec, respectively; $P < 0.05$). This result is interesting as hardness is affected by product expansion (Bordoloi and Ganguly, 2014). In general, a more expanded product would result in decreased hardness due to increase of cell openness. In this study, the starch content in the formulas likely affected the hardness of the kibbles. As the corn content increased in the LF treatments, the hardness of kibbles could have increased due to the cell wall strength formed by corn. Further, Jin et al. (1995) reported that thickness of cell walls and smaller air cells increased breaking strength, and thereby hardness. The IN treatments had the lowest peak force and compression area ($P < 0.05$). This could be attributed to the different phase of the cell walls driven by the different moisture content. Water has plasticization effect having transition from brittle (glassy) state to rubbery state.

There was no difference in moisture content for raw materials, off-preconditioner, or off-extruder samples among treatments. This indicates that all treatments had similar water holding capacity. However, NO treatments had the highest off-dryer moisture content (average; 11.42%) while the IN treatments had the lowest (average, 10.36%; $P < 0.05$). Overall, the moisture content of the dried products was less than 12%, which is typical for dry kibble products (Carrion and Thompson, 2014).

The peak viscosity indicates the maximum viscosity, the hold viscosity indicates the minimum viscosity, and the final viscosity relates to the retrogradation of materials after cooling down. The breakdown is the difference between the peak and the hold viscosity, the setback is the

difference between the final and the hold viscosity. Peak time provides an estimate of gelatinization time or speed of the treatment samples (Figure 3). The peak viscosity for EXHF was higher than that for INHF ($P < 0.05$), and the breakdown for EXHF was higher than that for INHF ($P < 0.05$) (Table 2.5). The addition of SBO might have coated the outside of the starch molecules preventing gelatinization and resulting in less cook within the extrusion, which could explain the higher peak viscosity.

The HF treatments had lower peak viscosity, breakdown, hold viscosity, final viscosity, and setback compared to the LF treatments ($P < 0.05$) (Table 2.6). Both EX and IN treatments had lower hold viscosity, final viscosity, final viscosity, but higher breakdown than NO treatments ($P < 0.05$). The decrease in viscosity might be due to the lower starch content in the HF treatments compared to the LF treatments. As starches are heated in an aqueous solution, they gelatinize increasing the viscosity of the solution (Brandt, 2003). Also, higher fat proportion in HF led to higher breakdown values by the fat lubrication. The higher breakdown value means stronger shear thinning characteristics in the starch polymer implying that the molecules are tended to be more aligned during continued heating and stirring during RVA testing. A high breakdown demonstrates the ease of starch granules to be broken upon heating after the maximum swelling at the peak viscosity (Rojas et al., 1999, Cornejo and Rosell, 2015).

2.4.4 Mass and energy balance

The thermal and total processing energy (specific thermal energy + specific mechanical energy; STE + SME) was greater for LF than HF (Figure 4). This might be due to higher steam retention within the extruder caused by the lower content of lipids in the LF diets. The discharge rate for LF (average 144.4 kg/h) was slower than HF treatments (average 150.4 kg/h). The thermal energy contribution was found to be higher than mechanical energy in most cases showing STE/

SME > 1.0 (Figure 5). Baller et al. (2021) reported that STE application is important for sufficient total specific energy implementation, enhancing kibble expansion, starch gelatinization, extruder productivity, and reducing SME application in dry foods for cats. The heat input from the extruder values (Q_{barrel}) were all negative across the treatments which implies that heat was lost from the material to the barrel, and not a transfer from barrel to material (Figure 6). The negative values of Q_{barrel} are possible since the material temperature (die temperature) was much higher than the maximum barrel temperature (90 °C). The EXLF and EXHF that contained soybean oil in the formula tended to have higher heat loss in the extruder barrel than the other treatments. Also, the heat loss from the extruder for 150 g/kg, as fed whole soybean inclusion treatments (NOLF, EXLF, and INLF) were lower than the 200 g/kg, as fed whole soybean inclusion treatments (NOHF, EXHF, and INHF). The surging within the single screw extruder might be attributed to higher loss of energy in the extruder barrel. When surging happens, materials stacked inside the barrel do not effectively interact with the friction heat. They could instantly increase the energy within the barrel and then come out in a larger volume at once.

To reach the realistic energy balance calculations, some of the assumptions that had to be made were adjusted. For instance, the negative value for steam loss from the preconditioner was clearly wrong since there was indeed steam loss due to the high discharge temperature (97 to 98 °C) from the preconditioner when the product was discharged. For another example, the negative value for heat loss from the preconditioner wall was impossible since we didn't provide any jacketed heat within the preconditioner to give extra heat energy into the raw materials. Therefore, in hindsight, we should have adjusted a couple of input values in the calculations.

Since we collected the off-preconditioner samples at the downspout of the preconditioner, the moisture content of the samples could have been underestimated due to the loss of vapored

water. To make sure the changes were 'consistent' across the treatments and matched with the trends from the original values for moisture content in off-preconditioner samples, we adjusted the moisture content of the off-preconditioner samples (X_{wpc}) to 23% for the first three treatment (NOHF, EXHF, INHF), and to 23.5% for the last three treatments (NOLF, EXLF, INLF). The steam out of the preconditioner and the extruder was low-pressure steam which is low-quality. In other words, it was not 100% steam, containing some water droplets. Therefore, we adjusted the steam quality for both preconditioner and extruder (q_{slpc} and q_{slex}) to 60%. The measured throughput values were also adjusted at the extruder to get 5% difference between the calibrated raw material feed rate and the calculated raw material feed rate. When we did feeder calibration, it was decided to use the same feeder rate for NOHF& NOLF and EXHF & EXLF, and INHF & INLF. Granted they are very similar in the formulas except for the addition of some degermed corn. Therefore, measured throughput values were adjusted from the extruder for EXHF to the same as that of EXLF.

2.5 Conclusions

The bulk density can be maintained with the addition of external or internal fat into the extruder by adjusting extruder shaft speed. However, the addition of external fat, soybean oil, resulted in unstable processing and inconsistent kibble qualities. This study suggests that internal fat inclusion in whole soybeans may be able to overcome the restriction of fat content in dry kibble. Further research needs to be done to determine how much internal fat can be added during extrusion.

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2.7 Chapter 2 Figures

30°C			70°C				90°C		
1	2	3	4	5	6	7	8	9	10
Inlet, single flight	Single flight	Spacer	Single flight	Spacer	Single flight	Small steamlock	Double flight	Medium steamlock	Cone, double flight

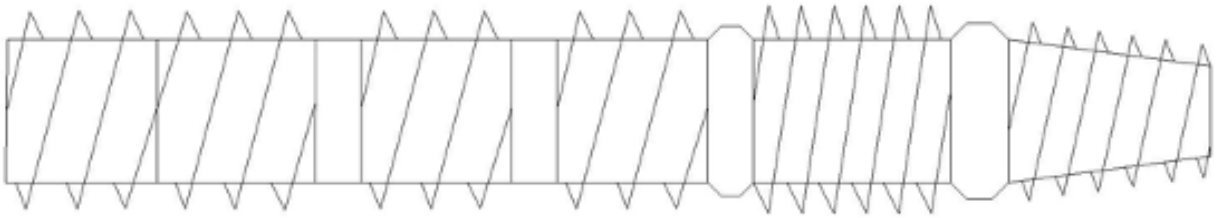

Figure 2.1 Screw profile for X-20.

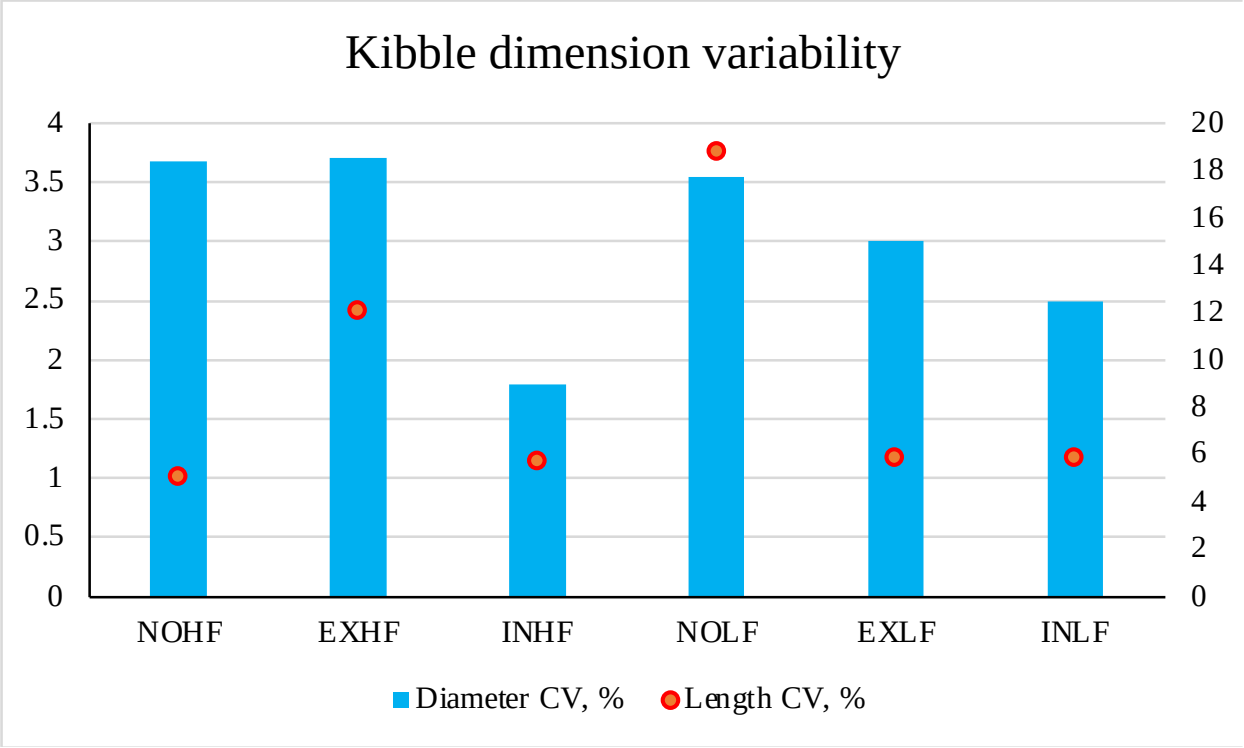

Figure 2.2 Kibble dimension variability indicated by coefficient of variation (%) of experimental diets containing NOHF (no high fat content), EXHF (high external fat inclusion), INHF (high internal fat inclusion), NOLF (no low fat content), EXLF (low external fat inclusion), and INLF (low internal fat inclusion).

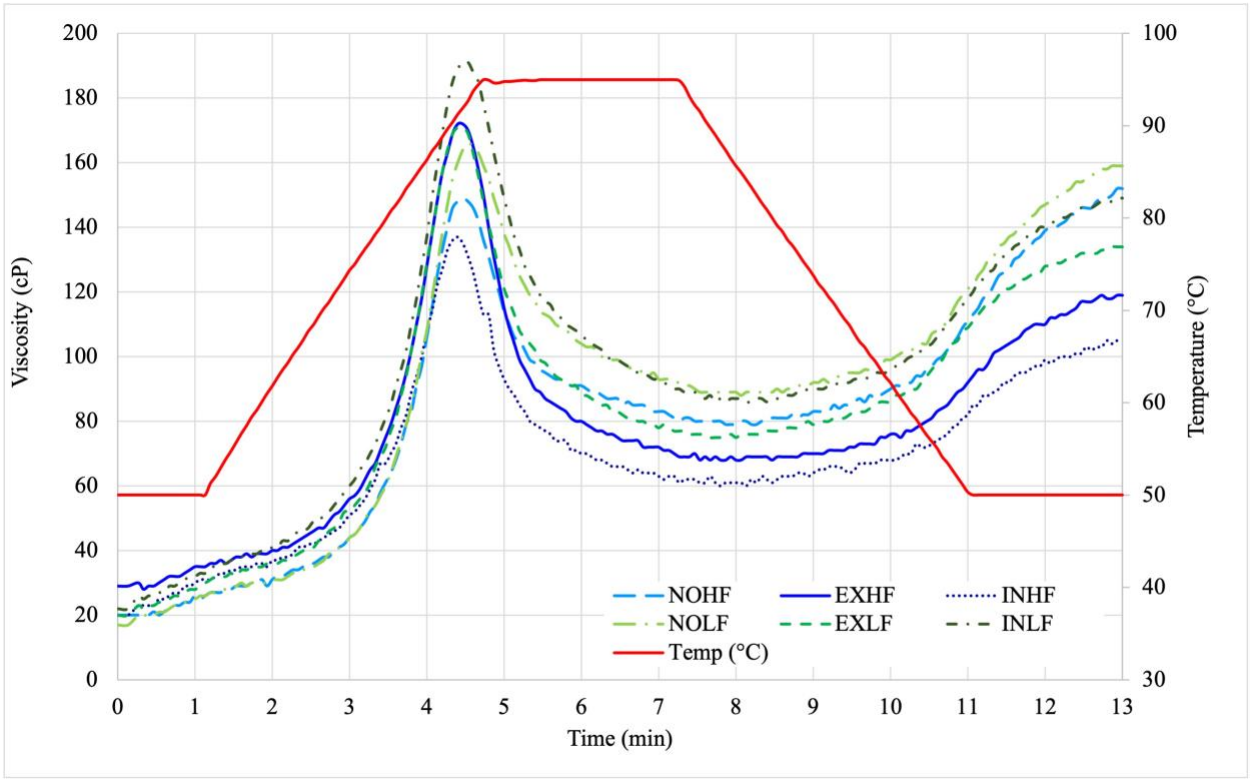

Figure 2.3 RVA profiles of representative replicate from each treatment; NOHF (no high fat content), EXHF (high external fat inclusion), INHF (high internal fat inclusion), NOLF (no low fat content), EXLF (low external fat inclusion), and INLF (low internal fat inclusion).

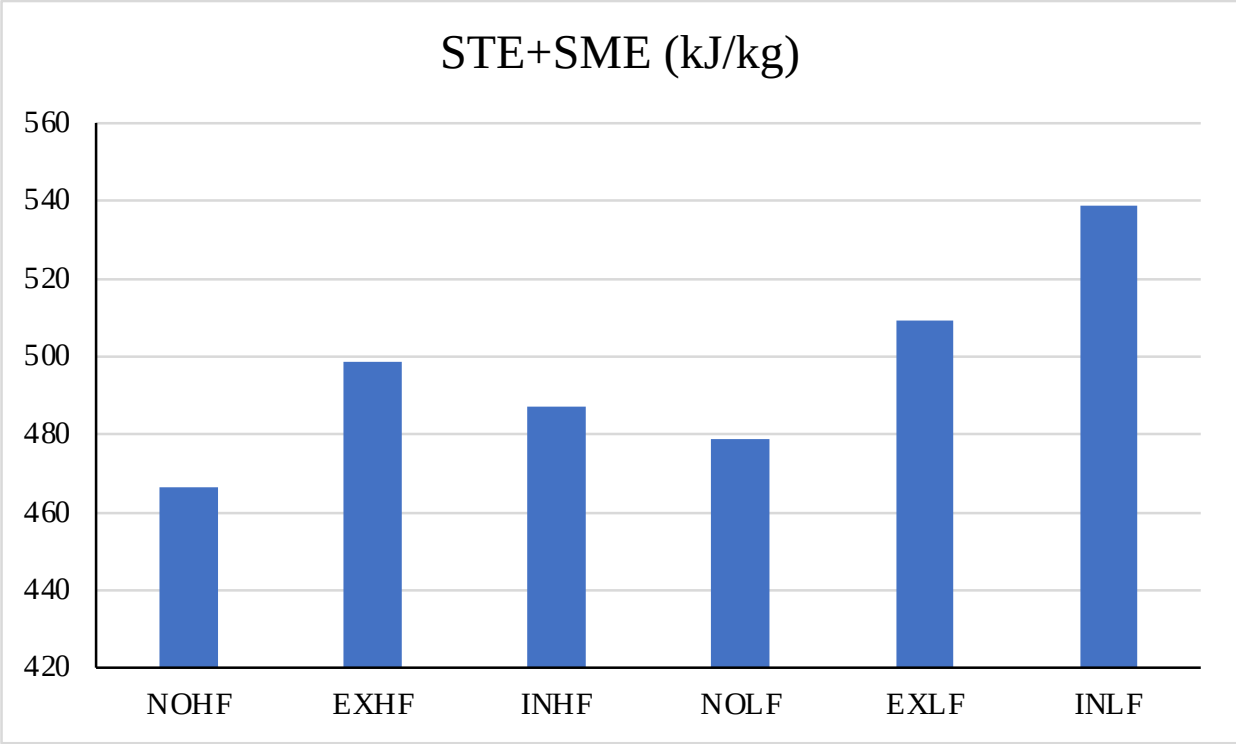

Figure 2.4 Total specific energy (STE+SME) (kJ/kg) of treatments containing NOHF (no high fat content), EXHF (high external fat inclusion), INHF (high internal fat inclusion), NOLF (no low fat content), EXLF (low external fat inclusion), and INLF (low internal fat inclusion).

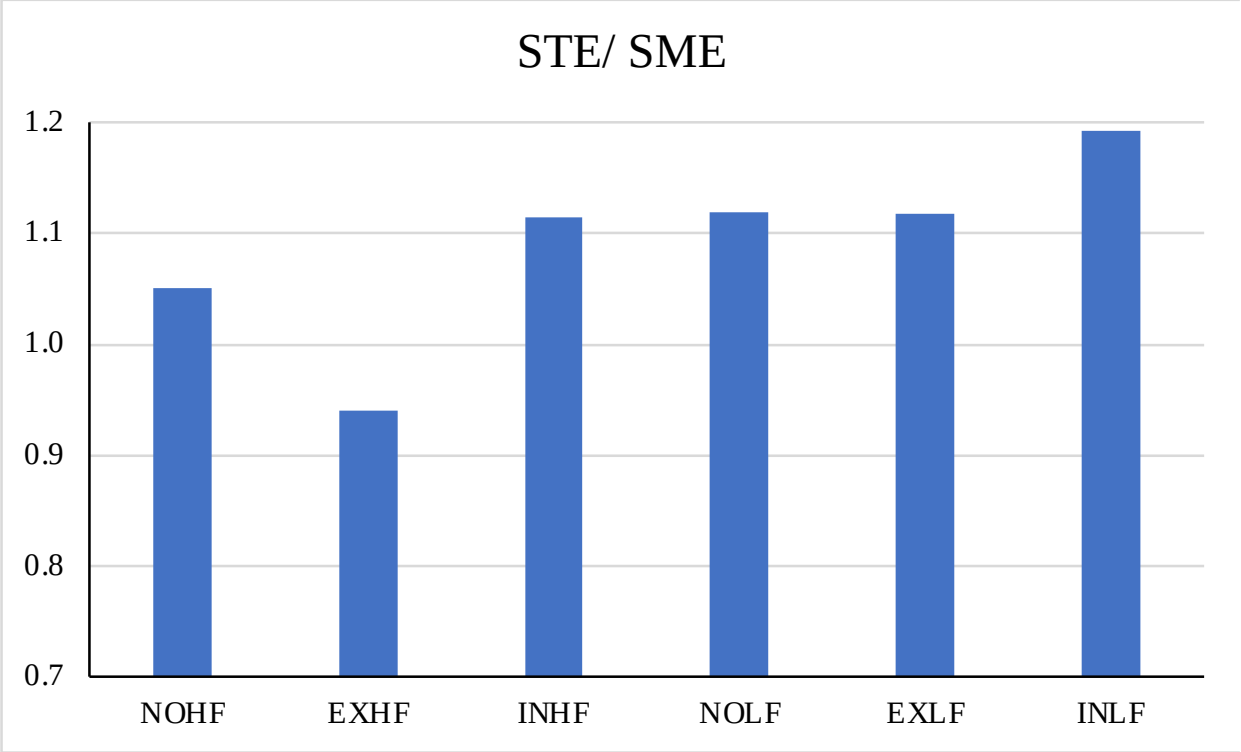

Figure 2.5 STE/ SME of treatments containing NOHF (no high fat content), EXHF (high external fat inclusion), INHF (high internal fat inclusion), NOLF (no low fat content), EXLF (low external fat inclusion), and INLF (low internal fat inclusion).

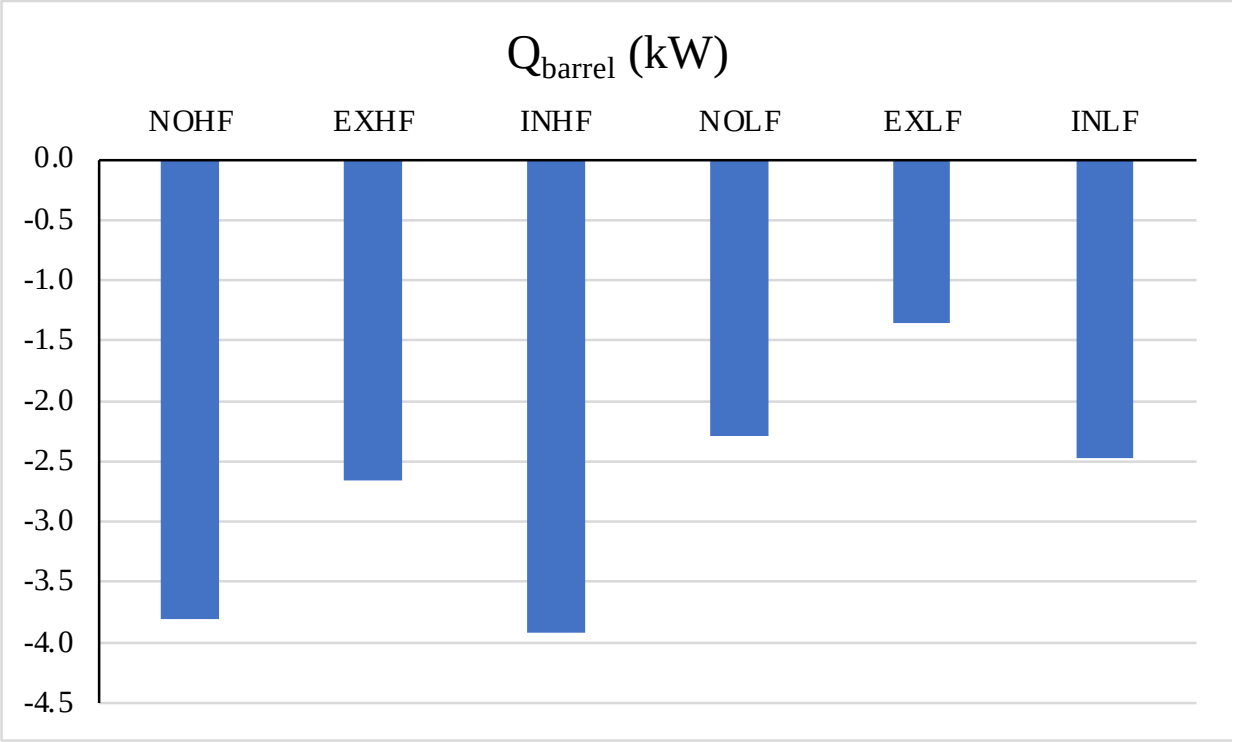

Figure 2.6 Q_{barrel} (kW) of treatments containing NOHF (no high fat content), EXHF (high external fat inclusion), INHF (high internal fat inclusion), NOLF (no low fat content), EXLF (low external fat inclusion), and INLF (low internal fat inclusion)

2.8 Chapter 2 Tables

Table 2.1 Diet formulas for the experiment.

Treatments	[1]HF			[2]LF		
	[3]NOHF	[4]EXHF	[5]INHF	[6]NOLF	[7]EXLF	[8]INLF
Ingredients, g/kg as fed						
[9]Base formula (BASE)	821.8	788.0	788.0	804.5	779.4	789.3
Soybean meal (SBM)	178.2	171.0	0.0	142.2	147.0	0.0
Whole soybeans (WSB)	0.0	0.0	212.0	0.0	0.0	158.0
Soybean oil (SBO)	0.0	41.1	0.0	0.0	31.5	0.0
Corn	0.0	0.0	0.0	53.3	42.1	52.7
Nutrient composition, g/kg (estimated, as-is basis)						
Dry matter	902.9	906.9	907.8	902.3	905.4	906.0
Crude protein	27.98	268.3	263.0	262.6	258.2	247.7
Crude fat	40.9	80.3	80.5	41.8	71.6	71.6
Crude fiber	30.3	29.1	31.9	29.7	28.9	30.9
Ash	70.9	68.0	68.4	68.4	66.7	66.8
Carbohydrate (NFE)	484.7	464.7	467.5	499.8	480.0	489.0

[1]HF = high fat content; [2]LF = low fat content; [3]NOHF = no high fat content; [4]EXHF = high external fat inclusion; [5]INHF = high internal fat inclusion; [6]NOLF = no low fat content; [7]EXLF = low external fat inclusion; [8]INLF = low internal fat inclusion; [9]Base formula (BASE) contains corn, wheat, chicken meal, brewer's rice, beet pulp, corn gluten meal, salt, dicalcium phosphate, titanium dioxide, potassium chloride, fish oil, choline chloride, calcium carbonate, vitamin premix, flaxseed, mineral premix, natural antioxidant.

Table 2.2 Processing parameters used to produce the experimental diets containing NOHF (no high fat content), EXHF (high external fat inclusion), INHF (high internal fat inclusion), NOLF (no low fat content), EXLF (low external fat inclusion), and INLF (low internal fat inclusion)

Treatments	[1]HF			[2]LF		
	NOHF	EXHF	INHF	NOLF	EXLF	INLF
Pre-conditioner						
Steam flow, kg/h	13.1	13.1	13.1	13.0	13.1	13.1
Water flow, kg/h	7.8	7.9	7.9	7.9	7.9	7.9
Discharge temperature, °C	98	97	98	97	97	97
Extruder						
Soybean oil rate, kg/h	0	5.1	0	0	3.8	0
Shaft speed, rpm	305	406	352	313	403	350
Steam flow, kg/h	0	0	0	0	0	0
Water flow, kg/h	15.5	14.2	13.5	15.8	15.1	15.3
Die temperature, °C	121	117-128	125-126	120-123	123-129	120-124
Die pressure, psi	300	300	300	350	300	300
Discharge rate, kg/h	143.4	159.8	148.1	149.7	143.7	139.9
Motor load, %	55	49-54	51-53	55-56	49-52	49-54
Motor power, kW	8.6	9.4-11.3	9.3	8.9-9.3	9.7-10.3	8.3-9.8

[3]SME, kJ/kg	229.7	264.7	240.1	244.4	254.3	232.8
[4]MC, %	29.7	28.3	28.9	29.8	28.9	29.6

[1]HF = high fat content; [2]LF = low fat content; [3]SME = specific mechanical energy; [4]MC = in-barrel moisture content.

Table 2.3 The least squares mean for kibbles dimensions, bulk density, texture analysis (compression test), and dry matter (DM) of experimental diets containing NOHF (no high fat content), EXHF (high external fat inclusion), INHF (high internal fat inclusion), NOLF (no low fat content), EXLF (low external fat inclusion), and INLF (low internal fat inclusion).

Treatments	[1]HF			[2]LF			[3]SEM	P-value		
	NOHF	EXHF	INHF	NOLF	EXLF	[8]NLF		Fat content	Fat insertion site	Interaction
[4]N	2	2	2	2	2	2				
Bulk density – raw rations, g/L	575.0	571.0	543.5	576.5	630.3	549.8	17.97	0.1788	0.0634	0.2789
Bulk density – off extruder, g/L	346.8	345	361.8	342.5	356.0	354.0	4.08	0.9236	0.0473	0.1259
Bulk density – off drier, g/L	335.0^{ab}	342.3^{a}	330.8^{b}	334.0^{ab}	334.5^{ab}	340.0^{a}	2.46	0.9366	0.3252	0.0365
[4]N	4	3	4	4	3	2				
Dried kibble diameter, mm	11.0	11.5	11.2	11.0	11.5	11.1	0.10	0.9136	0.0012	0.8796
Dried kibble length, mm	6.6^{ab}	6.8^{a}	6.4^{b}	6.7^{a}	6.4^{b}	6.3^{b}	0.09	0.0716	0.0247	0.0325
Dried kibble mass, g	0.31^{b}	0.42^{a}	0.31^{b}	0.32^{b}	0.33^{b}	0.31^{b}	0.02	0.0908	0.0064	0.0344
Specific length, cm/g	2.1	1.7	2.1	2.1	1.9	2.1	0.07	0.1901	0.0006	0.0655
[5]SEI	3.1	3.4	3.2	3.1	3.4	3.2	0.06	0.9649	0.0012	0.8887

[4]N	15	15	15	15	15	15				
Peak force, kg	15.95	16.33	13.92	18.20	17.68	17.10	0.63	<0.0001	0.0241	0.3541
Compression area, kg*sec	14.39	14.70	12.52	17.34	16.03	15.18	0.61	<0.0001	0.0041	0.3687
[4]N	2	2	2	2	2	2				
DM – off drier, %	88.41[d]	89.22[b]	90.08[a]	88.75[c]	89.05[b]	89.19[b]	0.05	0.0014	<0.0001	<0.0001

[1]HF = high fat content; [2]LF = low fat content; [3]SEM = standard error of the least squares means; [4]N = number of replicates; [5]SEI = sectional expansion index; [abcd]Within a row, means without a common superscript differ for the interaction (P < 0.05).

Table 2.4 Main effect (fat content or fat insertion site) least squares mean for kibbles dimensions, bulk density, texture analysis (compression test), and dry matter (DM) of experimental diets.

Treatments	Fat content			Fat insertion site				P-value		
	[1]HF	[2]LF	[3]SEM	[4]NO	[5]EX	[6]IN	[3]SEM	Fat content	Fat insertion site	Interaction
[7]N	6	6		4	4	4				
Bulk density – raw rations, g/L	563.2	585.5	10.37	575.8	600.6	546.6	17.97	0.1788	0.0634	0.2789
Bulk density – off extruder, g/L	351.2	350.8	2.36	344.6^{b}	350.5^{ab}	357.9^{a}	4.08	0.9236	0.0473	0.1259
Bulk density – off drier, g/L	336.0	336.2	1.42	334.5	338.4	335.4	1.74	0.9366	0.3252	0.0365
[7]N	11	9		8	6	6				
Dried kibble diameter, mm	11.2	11.2	0.06	11.0^{b}	11.5^{a}	11.1^{b}	0.07	0.9136	0.0012	0.8796
Dried kibble length, mm	6.6	6.5	0.05	6.7^{a}	6.6^{a}	6.4^{b}	0.06	0.0716	0.0247	0.0325
Dried kibble mass, g	0.35	0.32	0.01	0.31^{b}	0.38^{a}	0.31^{b}	0.01	0.0908	0.0064	0.0344
Specific length, cm/g	2.0	2.0	0.04	2.1a	1.8^{b}	2.1^{a}	0.07	0.1901	0.0006	0.0655
[3]SEI	3.2	3.2	0.04	3.1b	3.4^{a}	3.2^{b}	0.04	0.9649	0.0012	0.8887

[7]N	45	45		30	30	30				
Peak force, kg	15.40[B]	17.66[A]	0.37	17.07[a]	17.00[ab]	15.51[b]	0.45	<0.0001	0.0241	0.3541
Compression area, kg*sec	13.87[B]	16.18[A]	0.35	15.87[a]	15.36[a]	13.85[b]	0.43	<0.0001	0.0041	0.3687
[7]N	6	6		4	4	4				
DM – off drier, %	89.23[A]	89.00[B]	0.03	88.58[c]	89.13b	89.64[a]	0.05	0.0014	<0.0001	<0.0001

[1]HF = high fat content; [2]LF = low fat content; [3]SEM = standard error of the least squares means; [4]NO = no fat content; [5]EX = external fat inclusion; [6]IN = internal fat inclusion; [7]N = number of replicates; [AB, abc]Within a main effect (fat content or fat insertion site), means without a common superscript differ ($P < 0.05$).

Table 2.5 The least squares mean for RVA analysis data for the dried kibbles of experimental diets containing NOHF (no high fat content), EXHF (high external fat inclusion), INHF (high internal fat inclusion), NOLF (no low fat content), EXLF (low external fat inclusion), and INLF (low internal fat inclusion).

	[1]HF			[2]LF				P-value		
	NOHF	EXHF	INHF	NOLF	EXLF	INLF	[3]SEM	Fat content	Fat insertion site	Interaction
[4]N	2	2	2	2	2	2				
Peak viscosity, cp	147.5^{cd}	163.0^{bc}	137.5^{d}	170.0^{abc}	173.0^{ab}	193.5^{a}	4.28	0.0002	0.1620	0.0043
Hold viscosity, cp	82.5	67.0	62.5	93.5	76.5	91.0	3.58	0.0014	0.0103	0.0681
Breakdown, cp	65.0^{c}	96.0^{ab}	75.0^{bc}	76.5^{bc}	96.5^{ab}	102.5^{a}	4.12	0.0078	0.0021	0.0450
Final viscosity, cp	152.0^{a}	117.0^{b}	107.5^{b}	166.0^{a}	135.0^{ab}	161.0^{a}	5.83	0.0010	0.0032	0.0275
Setback, cp	69.5^{a}	50.0^{b}	45.0^{b}	72.5^{a}	58.5^{ab}	70.0^{a}	3.29	0.0039	0.0049	0.0361
Peak time, min	4.5	4.4	4.4	4.5	4.2	4.4	0.09	0.4795	0.1597	0.4199
Pasting temp, °C	92.4	91.3	90.9	92.5	88.9	91.4	1.09	0.4970	0.1768	0.4315

[1]HF = high fat content; [2]LF = low fat content; [3]SEM = standard error of the least squares means; [4]N = number of replicates; [abc]Within a row, means without a common superscript differ significantly ($P < 0.05$).

Table 2.6 Main effect least squares mean for RVA analysis data for the dried kibbles of experimental diets.

	Fat content			Fat insertion site				P-value		
	[1]HF	[2]LF	[3]SEM	[4]NO	[5]EX	[6]IN	[3]SEM	Fat content	Fat insertion site	Interaction
[7]N	6	6		4	4	4				
Peak viscosity, cp	149.3B	178.8A	2.4693	158.8	168.0	165.5	3.02	0.0002	0.1620	0.0043
Hold viscosity, cp	70.7B	87.0A	2.0683	88.0a	71.8b	76.8b	2.53	0.0014	0.0103	0.0681
Breakdown, cp	78.67B	91.83A	2.3776	70.8b	96.3a	88.8a	2.91	0.0078	0.0021	0.0450
Final viscosity, cp	125.5B	154.0A	3.3686	159.0a	126.0b	134.3b	4.13	0.0010	0.0032	0.0275
Setback, cp	54.8B	67.0A	1.8966	71.0a	54.3b	57.5b	2.32	0.0039	0.0049	0.0361
Peak time, min	4.4	4.4	0.0516	4.5	4.3	4.4	0.06	0.4795	0.1597	0.4199
Pasting temp, °C	91.5	90.9	0.6277	92.4	90.1	91.1	0.77	0.4970	0.1768	0.4315

[1]HF = high fat content; [2]LF = low fat content; [3]SEM = standard error of the least squares means; [4]NO = no fat content; [5]EX = external fat inclusion; [6]IN = internal fat inclusion; [7]N = number of replicates; [AB, ab]Means within a main effect (fat content or fat insertion site), without a common superscript differ ($P < 0.05$).

Chapter 3 - Extrusion and product parameters for extruded dog diets with graded levels of whole soybeans

3.1 Abstract

Increasing the fat amount of the ration during extrusion can negatively affect product density and product expansion. Ingredients, like whole soybeans (WSB), which are high in fat may increase energy density while avoiding production issues. The objective of this study was to determine the effect of WSB on extrusion and product characteristics of dry expanded dog food. Experimental diets were extruded with 0, 100, 200, and 300 g/kg, as fed WSB. The processing conditions during extrusion were kept constant across all treatments. Processing outputs, trypsin inhibitor, urease, and phytic acid concentrations were analyzed. Single degree of freedom contrasts were used for statistical analysis and significance at $\alpha = 0.05$. As WSB increased, the extruder load, die pressure and temperature, and specific mechanical energy (SME) decreased linearly ($P < 0.05$). The bulk density of the products increased linearly ($P < 0.05$) while the kibble sectional expansion index (SEI) decreased linearly ($P < 0.05$) as WSB increased. Post-extrusion, trypsin inhibitor and urease activity decreased, but trypsin inhibitor was not eliminated. In conclusion, the increased inclusion level of WSB changed processing conditions and outputs as a result of the intrinsic fat

content. Increasing energy input in processing may destroy anti-nutritional factors completely when WSB are included in diets.

3.2 Introduction

Soybeans are the most important oilseed crop grown in the United States, accounting for nearly 90% of U.S. oilseed production. Soybeans have excellent potential for delivering quality proteins as an ingredient. However, it has the undeserved reputation from social media outlets of being a poor quality ingredient in modern pet food. Some will claim that soy is of low nutritional quality relative to other protein sources, may lead to allergies, is a cheap by-product, and should be avoided due to genetically modified organisms (GMO) status.

Soybeans contain anti-nutritional factors including trypsin inhibitors (Félix et al., 2020), oligosaccharides (Stein et al., 2008), and phytate phosphorus. However, all but phytic acid and oligosaccharides can be deactivated through heat processing. Most other anti-nutritional factors are proteins in nature. For example, the trypsin inhibitors are a group of serine protease enzymes, and they reduce the biological activity of the digestive enzymes such as trypsin and chymotrypsin (Vagadia et al., 2017). Urease is an enzyme that decomposes urea to ammonia and carbon dioxide (Yalcin and Basman, 2015). In order to improve the nutritional value of soybeans, a heat treatment such as adding steam or extrusion cooking is used (Van den Hout et al., 1998). The heat during the extrusion denatures the enzyme protein structure and renders them inactive (Riaz, 2000; Nikmaram et al., 2015), thereby decreasing trypsin inhibitor and urease activity units (Purushotham et al., 2007; Drulyte and Orlien, 2019; Félix, et al., 2020). According to previous research with pet foods, soybean products have resulted in benefits to processing, nutrient utilization, acceptability,

and gut health (Yamka et al., 2005; Carciofi et al., 2009; Félix et al., 2013; Tortola et al., 2013; Menniti et al., 2014; Venturini et al., 2018). Previous research also has demonstrated that extrusion improved the bioavailability of soybean products in broilers, pigs, and dogs (Woodworth et al., 2001; Jahanian and Rasouli, 2016; Félix et al., 2020).

The U.S. pet industry had $109.6 billion in total expenditures in 2021 with pet food and treats accounting for $ 50.0 billion (American Pet Products Association, 2022). As this market has grown, the types of pet food and their categories have become more differentiated. One of the emerging requests from some consumers is a high-fat, high-energy-density dry dog foods (Dog Food Guide, 2022). However, the amount of fat that can be added to kibble without vacuum coating is limited in dry expanded pet food. First, if too much fat is applied by coating, the kibble may not absorb the fat sufficiently and lead to fat leaching onto the package during storage. Second, directly introducing the liquid fat into the ration during extrusion may negatively affect product expansion and bulk density due to lubrication. Fat reduces friction between the materials and extruder shaft within the extruder barrel resulting in difficulty in transmitting mechanical energy from the screws into the product (Lin et al., 1997). Fat also coats the feed particles preventing moisture absorption and thermal heat transfer. It may hinder the materials from being fully cooked or prevent starch from being fully gelatinized during extrusion (Rokey et al., 2010). However, intrinsic fats included as a component of an ingredient may have less effect on product expansion than added fat (Rokey et al., 2010). Therefore, it was hypothesized that ingredients like whole soybeans (WSB) that are high in internal fat may increase energy density while avoiding processing limitations.

Information is lacking on the effect of supplementation of WSB on typical pet food processing conditions and final product characteristics. Therefore, the objective of this study was to determine the effect of WSB on extrusion and product parameters for dry expanded dog food.

3.3 Materials and methods

3.3.1 Experimental diets

Four experimental diets were formulated to be nutritionally adequate for adult dogs (AAFCO, 2020). The corn gluten meal, chicken fat, and brewers rice were replaced by WSB at 100, 200, and 300 g/kg, as fed (WSB100, WSB200, and WSB300, respectively) in the base diet (WSB0) (Table 3.1). Diets were formulated to have similar macro- and micronutrient composition and included titanium dioxide (TiO2; 4 g/kg, as fed as an indigestible marker for later use in a dog feeding study. As predicted by the formulation, the concentration of minerals - calcium, phosphorus, potassium, magnesium, sodium, sulfur, manganese, copper iron, and zinc were similar among diets and met AAFCO recommendations for adult dogs at maintenance (AAFCO, 2020).

Raw materials for the base ration were blended by a commercial mill (Fairview Mills, Seneca, KS, USA) with particle size reduced via hammer mill to pass through a 2 mm screen. Whole soybeans were provided by MKC (Manhattan, KS, USA), and were cleaned using a grain cleaner and ground with a hammer mill (model 18-7-300; Schutte Buffalo, NY, USA) to pass through a 1.19 mm screen (3/64") at the Hal Ross Flour Mill (Manhattan, KS, USA). All ingredients were mixed in a paddle mixer (136 kg capacity) for 5 min. The chicken fat and digest (dry dog flavor) were added topically to kibbles after extruding and drying.

3.3.2 Extrusion processing

The dry expanded pet foods (Figure 1) were produced using a single screw extruder (EX; model E525, ExtruTech, Inc., Sabetha, KS, USA). The pre-conditioner (PC; model ADP 145, ExtruTech, Inc., Sabetha, KS, USA) was configured with 12 beaters 45 ° back, and 57 beaters in the neutral position, on each of two shafts, and operated at 185 rpm (Figure 2). The extruder had a defined profile (Figure 3) and barrel temperatures based on a typical commercial pet food configuration. At the end of the extruder barrel there were two round die inserts with interior diameter of 9.5 mm. The target in-barrel moisture was approximately 25% within a range between 23% and 27% wet basis. Fixed input parameters were kept constant throughout all food production and included dry matrix feed rate (431 kg/h), PC cylinder speed (185 rpm), PC water (59 ± 0.2 kg/h), PC steam (53 ± 0.3 kg/h), extruder (EX) water (0%), EX steam (0%), EX screw speed (425 rpm), and EX knife speed (700 rpm).

During experimental diet processing, PC and EX parameters were all collected from sensor readouts every 5 minutes to estimate the effects of different inclusion levels of WSB on extrusion. Output variables were those parameters resulting from the input variables, and included PC discharge temperature, EX die temperature and pressure, EX motor load, specific mechanical energy (SME), and total mass flow (TMF). Measurements were collected at uniform time increments (15 min term) during the production for a total of 3 times per experimental diet and were considered treatment replicates.

After extrusion, the products were conveyed pneumatically through an 8” Clean Air Hood System (KS, USA) and deposited onto an oscillating belt spreader that spread the kibbles evenly across the dryer bed. The kibbles were dried on a 5 ft wide single pass two zone dryer (model AFI, ExtruTech, Sabetha, KS, USA) to achieve the moisture content of

kibbles below 9%. The dryer was set at 109 ± 5.9 °C throughout the entire treatment process, and kibble retention time was 24 min. After drying, the chicken fat and digest (dry dog flavor) were applied to the kibbles in a ribbon mixer (136 kg capacity). Samples of kibbles before and after coating were collected for further analysis.

The TMF was calculated by adding the dry feed rate with water and steam injected in PC and EX, assuming that 80% of the water coming from the PC and EX steam is lost during flash-off, as kibbles exit the die:

TMF = dry feed rate + PC water + (0.2 * PC steam) + EX water + (0.2 * EX steam)

SME was calculated using the following formula:

$$\text{SME (kJ/kg)} = \frac{\frac{(\tau - \tau 0)}{100} * \frac{N}{Nr} * Pr}{m}$$

where, τ is the EX % torque or EX motor load, τ0 is the EX no load % torque (25% at EX screw speed 425 rpm), N is the EX screw speed (rpm), Nr is the rated EX screw speed (425 rpm), Pr is the rated EX motor power (114 kW), and m is TMF (kg/s).

3.3.3 Physical characteristics

Five kibbles from each time period (every 15 min) of each diet production off the extruder and off the dryer were randomly selected. Using a digital caliper, kibble diameter and length were measured. Five random kibbles off the dryer were weighed using a digital scale with 0.0001 g sensitivity (Explorer EX324N, Ohaus Corporation, Parsippany, NJ, USA.). The diameter, length, and mass measurements were used to determine sectional expansion index, specific length, piece volume, and density.

Sectional expansion index (SEI) was determined by comparing the squared diameter of the dried extruded kibbles by the squared die diameter of the extruder:

$$SEI = \frac{(D)\text{^}2}{(d)\text{^}2}$$

where D is the extrudate diameter and d is the extruder die diameter.

Specific length in cm/g was determined by the following equation:

$$Specific\ length = \frac{l}{m}$$

where, *l* is the extrudate length and *m* is extrudate mass.

Piece volume in cm^3 was determined by the following equation:

$$Piece\ volume\ (V) = \frac{\pi\ D\text{^}2 l}{4}$$

where, *D* is the extrudate diameter and *l* is extrudate length.

Piece density in g/cm^3 was determined by the following equation:

$$Piece\ density = \frac{m}{V}$$

where, *m* is the piece mass and *V* is piece volume.

Wet bulk density was measured off the extruder in three replicates manually during each treatment processing using a 1 L cup and leveling the kibbles with a metal ruler and weighing on a digital scale with 0.1 g sensitivity. Dry bulk density was measured off the dryer in three replicates using the same method.

3.3.4 Chemical analysis

All chemical analysis was performed in duplicate unless otherwise specified. The WSB and diets were ground using a fixed blade laboratory mill (Retch, type ZM200, Haan, Germany) fitted with a 1.0-mm screen and stored in lidded glass jars in preparation for chemical analysis. The ground WSB and experimental diets (after coating) were analyzed for dry matter (DM), organic matter (OM), and ash according to methods of Association of Official Analytical Chemists (AOAC, 2019; methods 934.01 and 942.05) (AOAC, 2019).

Crude protein (CP) content of the samples was determined by the Dumas combustion method (AOAC 990.03) using a nitrogen analyzer (FP928, LECO Corporation, Saint Joseph, MI, USA) (AOAC, 2019). Acid hydrolyzed ether extract (AHEE) was determined by acid hydrolysis (AOAC 964.02). Gross energy (GE) was determined by bomb calorimetry (Parr 6200 Calorimeter, Parr Instrument Company, Moline, IL, USA). Total starch (TS) content of the samples was determined following the standard procedure from the Total Starch Assay Kit (K-TSTA-100A, Neogen, Lansing, MI, USA). Total dietary fiber (TDF) content of the samples was measured following the standard procedure from the Total Dietary Fiber Assay Kit (K-TDFA-200A, Neogen, Lansing, MI, USA). The WSB, rations, and dried extrudate samples (off the dryer) were sent to a commercial analytical laboratory (Agricultural Experiment Station Chemical Laboratories, Columbia, MO, USA) to determine phytate, urease activity and trypsin inhibitor activity. Phytate was analyzed according to AOAC 986.11 (AOAC, 2019). Urease activity and trypsin inhibitor activity were analyzed according to AACC international approved methods of analysis (AACC, 2006; methods 22-90 and 22-40 (AACC, 2006).

3.3.5 Statistical analysis

The diets were produced in the order of WSB0, WSB10, WSB20, and WSB30 without randomization to ensure that control over the energy input into the raw materials was the same throughout all treatments during processing. For each dietary treatment, sampling was conducted at evenly spaced intervals (15 min) and those were considered replicates for the purpose of determining variability and control during production. Least square means of extrusion output responses and antinutritional factors were estimated by ANOVA using the GLM procedure with SAS (2013) using Tukey correction. Contrasts comparing “control

(WSB0) vs. treatments (WSB100, WSB200, and WSB300)," linear, quadratic, and cubic relationships of diets with graded level of WSB were considered significant at $P < 0.05$.

3.4 Results

3.4.1 Whole soybeans and experimental diets

The whole soybeans in this experiment contained 380 g/kg CP, 210 g/kg AHEE, and 200 g/kg TDF on a dry matter basis (Table 3.2). The diets were slightly drier than target (9%), but well within normal production parameters to avoid molding. The diet formulation was to maintain similar levels of protein and fat, and this was met except for the WSB replacement. Due to the increasing amount of WSB replacement for corn gluten meal and rice from 0 to 300 g/kg, as fed, CP linearly decreased ($P < 0.05$), while AHEE and GE linearly increased ($P < 0.05$) in uncoated kibbles (Table 3.3). These linear relationships continued through to coated kibbles, and due to decreasing fat coating, the GE for coated kibbles was not different among treatments.

3.4.2 Extrusion processing

Since the goal was to determine the effect of WSB inclusion levels, the input variables during extrusion were kept constant across all treatments (Table 3.4). Specifically, the PC cylinder speed, PC water, and EX screw speed (which can influence mechanical energy input), and PC steam (which can influence thermal energy input) were kept constant throughout all dietary treatment production cycles.

The PC discharge temperature was lower in the control (WSB0) compared to WSB treatments and increased linearly ($P < 0.05$) as WSB inclusion increased (Table 3.5). The extruder motor load, extruder die temperature, and extruder die pressure were higher in WSB0 compared to WSB-containing treatments and decreased linearly ($P < 0.05$) within

higher WSB levels. The SME was greater in WSB0 than for the other dietary treatments, and it decreased linearly ($P < 0.05$) as WSB inclusion increased. The TMF during all treatment productions was maintained within a very narrow range (499.9-500.5 kg/h).

3.4.3 Physical characteristics

The wet bulk density was lower in WSB0 compared to treatments and increased linearly ($P < 0.05$) as WSB inclusion increased (Table 3.6). The target wet kibble diameter (off the extruder) was 14 mm and the length was 6.5 mm in order to accommodate a future animal feeding study. These dimensions were achieved with similar wet kibble size throughout the treatments. The wet kibbles were less expanded as the WSB level increased ($P < 0.05$) with smaller kibble diameter and piece SEI off the extruder. The same relationship was observed with dried kibbles off the dryer, wherein the dry bulk density was also lower in WSB0 compared to treatments and increased linearly ($P < 0.05$) as WSB inclusion increased. This corresponded to a linear decline ($P < 0.05$) in kibble diameter, kibble length, piece volume, specific length, and piece SEI off the dryer, meaning a less expanded product as higher quantities of WSB were included in diets. The dried kibble mass was maintained relatively constant across the treatments. However, the piece density for WSB0 was lower than for treatments and it increased linearly ($P < 0.05$) at higher WSB inclusion levels.

3.4.4 Antinutritional factors

The raw WSB used in this study contained antinutritional factors such as trypsin inhibitor, urease and phytic acid (Table 3.7). The trypsin inhibitor and urease activity of raw rations increased linearly ($P < 0.05$) as the WSB inclusion level increased, with the lowest values for WSB0. The phosphorus and phytic acid contents did not differ among treatments. After the extrusion and drying process, there was a substantial reduction in both trypsin

inhibitor and urease activity compared to the raw rations but, the reduction was proportionally different as the WSB inclusion levels increased. The trypsin inhibitor decreased 25% for WSB0, 68% for WSB100, and 78% for both WSB200 and WSB300. The urease activity decreased 67% for WSB, 78% for WSB100, 95% for WSB200, and 97% for WSB300. The linear decrease ($P < 0.05$) in trypsin inhibitor and urease activity at higher WSB level was similar after processing. The phosphorus and phytic acid contents, however, were unaffected by extrusion and drying process.

3.5 Discussion

3.5.1 Whole soybeans and experimental diets

Félix et al. (2020) reported that raw soybeans used in extruded pet foods contained 401 g/kg CP, 209 g/kg acid hydrolyzed fat, 70 g/kg crude fiber, and 5,797.1 kcal/kg (DM basis). The crude fiber measurement grossly underestimates the TDF content of diets (Farcas et al., 2013). The TDF content (198 g/kg; dry matter basis) of the WSB in this study more accurately reflects the true fiber present in the ingredient. WSB are known to to contain approximately 80 g/kg, as fed soybean hulls (Kim et al., 2015; Kim et al., 2021), and soybean hulls contain from 600-800 g/kg, as fed TDF. Detweiler et al. (2019) incorporated soybean hulls into experimental diets that resulted in values of 143.0 g/kg, as fed for TDF, 124.0 g/kg, as fed for insoluble dietary fiber (IDF), and 19 g/kg, as fed for soluble dietary fiber (SDF).

Since WSB are considered as a protein, starch, and fat source, corn gluten meal, brewers rice, and chicken fat were decreased as necessary in WSB100, WSB200, and WSB300 to maintain predicted consistent nutrient levels across experimental diets. However, the actual TS and GE decreased at the higher WSB inclusions. This is because of the lower inclusion levels of brewer's rice that is mainly starch and low in fiber.

3.5.2 Extrusion processing

This study was intended to evaluate WSB as a means of increasing the energy density of dry expanded dog foods. Collecting the processing information was vital to fully understanding how WSB affect the process and finished product during extrusion of pet foods. To clearly determine the effect of WSB on extrusion outputs, input variables were controlled with little or no fluctuation. The linear increase in PC discharge temperature at higher WSB levels was within a narrow range across treatments and was interpreted to be of no practical importance.

The fat content of the rations entering the extruder varied due to the different inclusion levels of WSB. With the higher internal fat content derived from the WSB, the frictional and shear forces produced within the EX decreased as WSB inclusion levels increased. Fat may have been released from the WSB cells, and some might have become free oil within the EX due to the cooking. Fats serve to lubricate both the interacting particles in the dough mass and the particles that are rubbing against the EX screw surfaces and EX barrel (Guy, 2001). It was reported that lipid levels over 5-6% impaired EX performance (Riaz, 2000) and feed fat levels higher than 5% hindered continuous flow in the single-screw EX (Park et al., 1993). Because fat makes materials slip within the barrel and lowers friction, the EX motor load decreases, and product expansion decreases due to insufficient pressure development during extrusion (Riaz, 2000).

The EX load (torque) is related to the viscosity of the feed material in the screw channel (De Pilli et al., 2011). Fats reduce the viscosity of the mixed dough inside the extruder by lubrication (Grenus et al., 1993). As the viscosity of the dough decreased due to the increased fat, the dependent variable EX load decreased. These theories were validated

by the linear decrease in EX load, EX die temperature, EX die pressure, and SME in this study. As a result of the control in water and steam input in PC and EX throughout the production, TMF remained within a narrow range. Reddy and Reddy (2015) suggested that the high fat content of oilseeds may limit thermal and mechanical effects by reducing the shearing forces and heat increase inside the extruder.

3.5.3 Physical characteristics

The experimental diets were produced successfully to target finished product characteristics and were deemed acceptable for feeding to dogs in a subsequent animal trial. Extruded kibbles with graded levels of WSB had a consistent, stable production, and had similar size among treatments. Increasing inclusion of WSB decreased kibble expansion, resulting in increases in both wet and dry bulk density, and decreases in both wet and dry piece SEI. These results are consistent with the previous research by Park et al. (1993) in which increasing fat content lowered expansion ratio and increased the bulk density.

In general, higher fat levels resulted in decreased expansion of the extrudate (Park et al., 1993). Rokey (2006) reported that a 1% increase in fat between 12-17 % increased the bulk density of the final product by 16 g/L. It is because fats largely have an impact on the processing of starch by preventing moisture absorption and thermal heat transfer and reduce the degradation of the starch polymer, resulting in lower starch expansion (Guy, 2001). In this study, the calculated fat content of the ration that went into the extruder were approximately 4.2, 6.2, 8.0, and 9.8% for WSB0, WSB100, WSB200, and WSB300, respectively. The increases in wet bulk density among treatments were 52 ± 2.1 g/L and those for dry bulk density were 56 ± 7.1 g/L. The actual increases in bulk density were higher than

what was proposed by Rokey (2006), and this may have been due to the amount of fiber found in WSB.

The WSB used in this study contained 197.3 g/kg TDF (dry matter basis). According to the Guy Classification System (Guy, 2001), fibers are dispersed phase fillers and known to have very poor functionality in extrusion, meaning they lead to less expanded final products. Fibrous ingredients have high hydration properties and tend to increase the bulk density of the products and often require different extruder configurations and processing conditions to process properly (Rokey et al., 2010). Although wet kibble dimensions seemed similar when producing the diet, the kibbles at higher WSB inclusion expanded less and had higher density due to the nature of WSB.

3.5.4 Antinutritional factors

The antinutritional factor contents for soybean slightly varied from other reports in the literature. For example, Félix et al., (2020) reported that the trypsin inhibitor was 15.91 mg/g and urease activity was 1.86 net pH increase on as-fed basis of raw soybean for dogs. According to Purushotham et al. (2007), the trypsin inhibitor was 51 mg/g and urease activity was 2 net pH increase for raw soybeans. The raw WSB used in this study contained 16.65 mg/g of trypsin inhibitor and 2.17 net pH increase of urease activity, which were in between of the previously reported values. The trypsin inhibitor and urease activity increased linearly as the WSB inclusion increased in the raw diets (rations). Interestingly, the reduction rate of trypsin inhibitor numerically increased from 68% at WSB100 to 78% at WSB300 as the WSB inclusion levels. Romarheim et al. (2005) reported approximately 76% reduction in trypsin inhibitor for diets containing 29% of defatted soybean meal or 29% of defatted soy flakes after extrusion, and it is consistent with the 78% reduction of trypsin

inhibitor in the diet containing 30% WSB in the present study. In the study by Félix et al. (2020), the reduction rate of trypsin inhibitor was in a range of 35% to 73% without showing linear trends in the increase of raw soybean content in the diets from 6 to 30%.

Various processing methods have been used to inactivate trypsin inhibitor in soy. These treatments have mainly included heating, pH adjustment, hydrolysis, and high pressure (Vagadia et al., 2017). Level of thermal inactivation of trypsin inhibitor is influenced by temperature, moisture content, and treatment duration (Žilić et al., 2012). Forced conventional drying and roasting are common heat treatments used for the elimination of trypsin inhibitors. Conventional hot air drying at 100 °C for 20 hours reduced the trypsin inhibitor activity by 80% (Agrahar-Murugkar and Jha, 2010). For roasting, a dryer temperature varying between 110 and 170 °C, can inactivate the trypsin inhibitor activity up to 85% (Vagadia et al., 2017). With heat treatment in an oven at 200 °C for 20 min, the activity of trypsin inhibitors from whole soybean flour was significantly reduced having a minimum residual of trypsin inhibitors rate (32.67%) (Andrade et al., 2016). The extruder die temperature and pressure of this study was 101 ± 1.4 °C and 256 ± 51.5 psi, resulting in the residual of trypsin inhibitor rate average at 37.81%.

Konstance et al. (1998) extruded various blends of soy products with cornmeal through a twin screw extruder at three extruder temperatures (100, 115, and 130 °C), and antitrypsin activity was not detected after extrusion of any blend. They concluded that the inactivation of trypsin inhibitors in soy products can be accomplished through the extrusion heat treatment. Purushotham et al. (2007) extruded pet foods containing 150 g/kg of raw soybeans and reported that the trypsin inhibitors were inactivated to desired levels (< 2.0 mg/g) in diets when the extrusion temperature range was at 125 to 140 °C. They found slower

inactivation of trypsin inhibitor activity units at 40 to 80 °C; however, the inactivation was increased with an increase in extrusion temperature from 80 to 140 °C.

On the other hand, Félix et al. (2013) reported that trypsin inhibitor of raw soybeans decreased after extrusion, which was controlled to maintain the density 430 to 480 g/L, but did not completely disappear. Moreover, Félix et al. (2020) reported that an extrusion temperature range between 115 and 138 °C using a single-screw extruder did not completely inactivate trypsin inhibitors of the diets containing raw soybeans from 60 to 300 g/kg of the diet. The complete elimination of trypsin inhibitors from the WSB-containing diets in the current study was not achieved. It might be attributed to the lower extrusion temperature (101 ± 1.4 °C) used to produce the diets. Furthermore, the die temperature and SME in the extruder linearly decreased due to the fat from WSB, since the processing conditions were kept constant across treatment production. Higher extrusion temperature might have been required for the dog diets that contained higher amount of WSB, the source of the trypsin inhibitor. Use of higher mechanical or thermal energy input into the diet production process to increase the extrusion temperature may permit complete inactivation of trypsin inhibitors in WSB included dog diets.

The urease activity value was effectively reduced in all diets after extrusion. Urease is more heat-sensitive than trypsin inhibitors. The urease activity values for processed kibbles (0.01 - 0.07) were consistent with those considered optimal for heat-processed soybean products (below 0.20) (Butolo, 2002). There have been research reports that indicated extrusion reduced urease activity values (Willis, 2003; Wiriyaumpaiwong et al., 2004; Purushotham et al., 2007; Félix et al., 2013; Félix et al., 2020). There was no difference among the treatments in phosphorus or phytic acid contents, and this is because we aimed to

meet the concentration of minerals for all diets according to AAFCO (2020) recommendations for adult dogs at maintenance. The phytate content remained the same after processing as expected. Phytate cannot be inactivated by heating (Zhang and Laflamme, 1999; Stein et al., 2008).

3.6 Conclusion

The inclusion level of whole soybeans changed processing conditions as a result of the intrinsic fat content. The inclusion level of whole soybeans up to 300 g/kg was easily accomplished in a single-screw extruder for expanded dog foods with piece size and density changing. The extrusion temperature at 101 °C reduced some antinutritional factors but was insufficient to eliminate the trypsin inhibitors in whole soybeans containing diets. Further research is needed to find optimal processing conditions for different inclusion levels of whole soybeans in dog diets to increase the bioavailability of soybeans. Considering the nutritional composition of whole soybeans, utilizing them as excellent sources of protein and fat in dog foods has economic potential.

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3.8 Chapter 3 Figures

Figure 3.1 Dog kibbles produced containing graded levels of whole soybeans (WSB0, WSB100, WSB200, and WSB300, respectively from the left to the right).

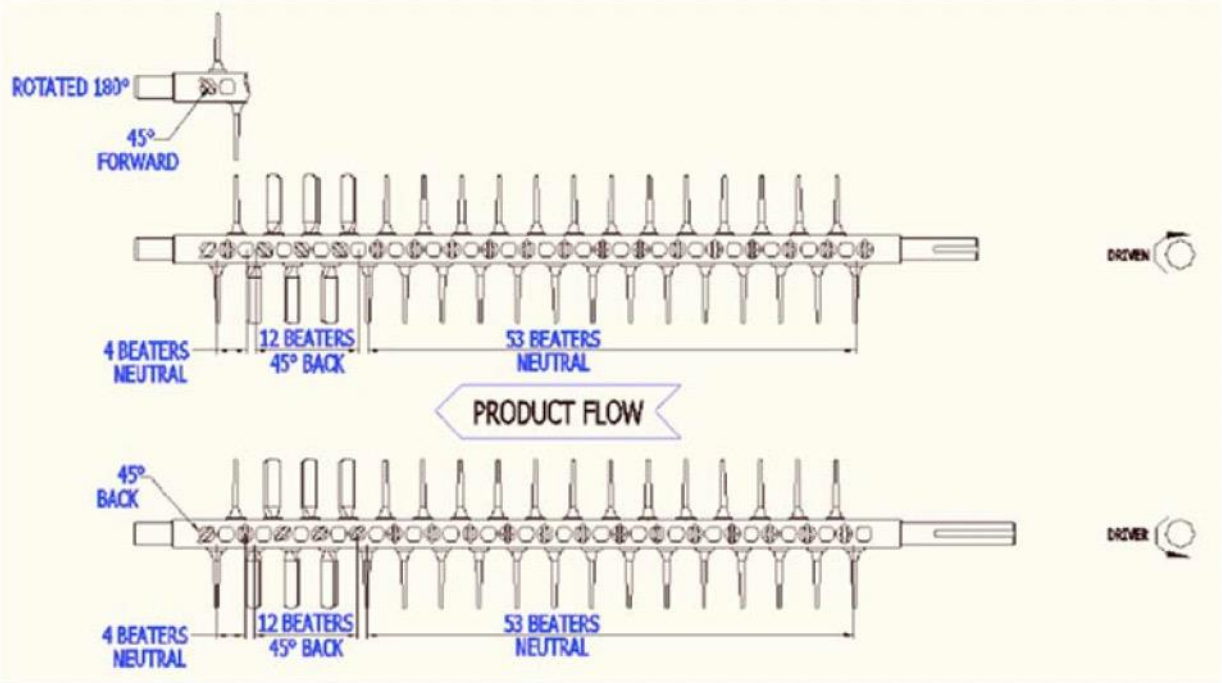

Figure 3.2 ADP 145 pre-conditioner beater configuration used to produce WSB0, WSB100, WSB200, and WSB300 diets.

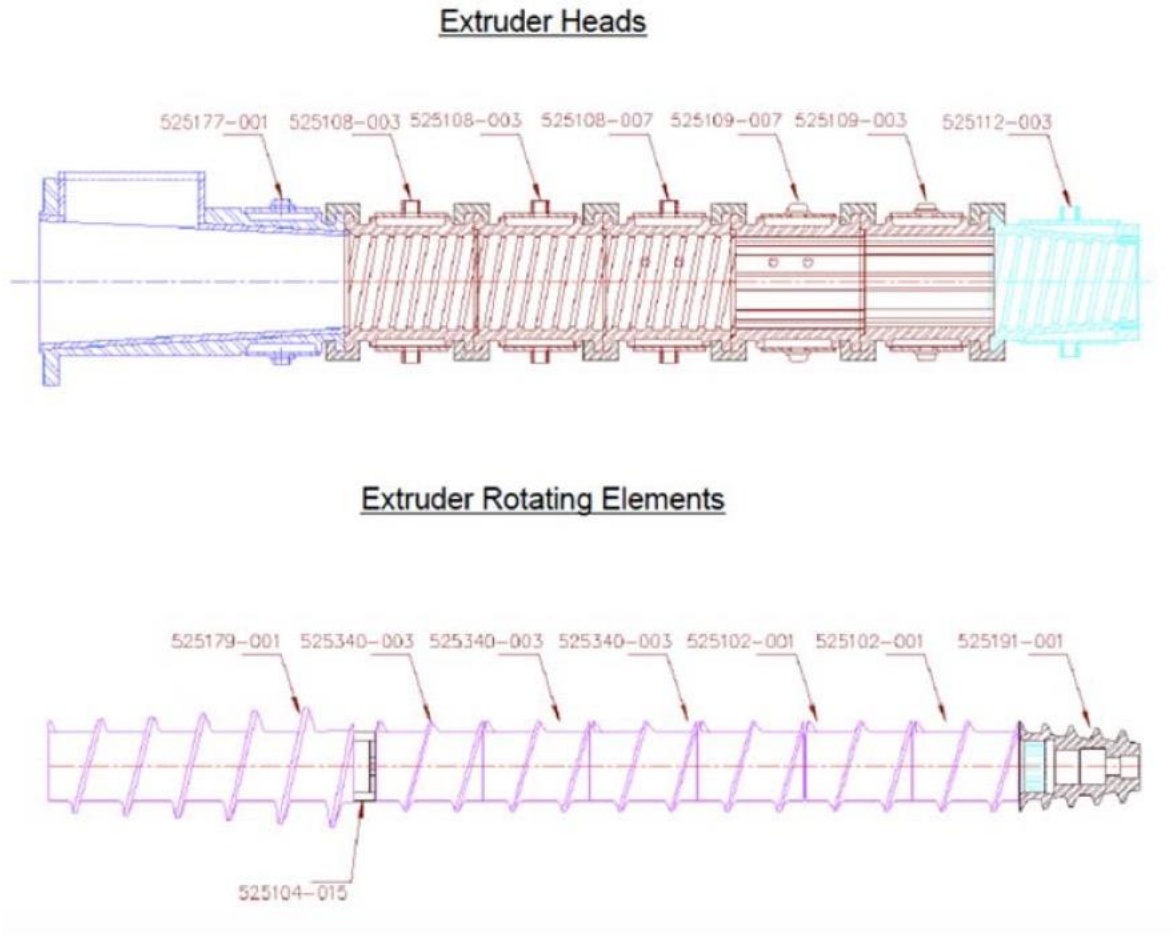

Figure 3.3 Extruder barrel configuration used to produce WSB0, WSB100, WSB200, and WSB300 diets.

3.9 Chapter 3 Tables

Table 3.1 Ingredient composition of diets with increasing levels of whole soybeans (WSB) (WSB0, 0 g/kg, as fed; WSB100, 100 g/kg, as fed; WSB200, 200 g/kg, as fed; and WSB300, 300 g/kg, as fed)

Ingredient, g/kg, as fed	WSB0	WSB100	WSB200	WSB300
WSB	0.0	100.0	200.0	300.00
Corn	225.0	225.0	225.0	225.0
Wheat	225.0	225.0	225.0	225.0
Corn gluten meal, 60 %	157.4	95.4	35.5	0.0
Chicken meal	150.0	150.0	150.0	150.0
Brewer's rice	85.8	66.7	45.4	0.0
Beet pulp	40.0	40.0	40.0	40.0
Salt	5.0	5.0	5.0	5.0
Dicalcium phosphate	4.5	4.5	4.5	4.5
Titanium dioxide	4.0	4.0	4.0	4.0
Potassium chloride	2.5	2.5	2.5	2.5
Choline chloride, 60 % dry	2.0	2.0	2.0	2.0
Fish oil	2.0	2.0	2.0	2.0
Calcium carbonate	1.5	1.5	1.5	1.5
Vitamin premix[1]	1.5	1.5	1.5	1.5
Flaxseed	1.3	1.3	1.3	1.3
Trace mineral premix[2]	1.0	1.0	1.0	1.0
L-Threonine 98 %	1.0	1.0	1.0	1.0
Dry natural antioxidant[3]	0.4	0.4	0.4	0.4
Chicken fat (topical)	80.2	61.3	42.5	23.4
Digest - dry dog flavor (topical)	10.0	10.0	10.0	10.0

[1]Vitamin premix per kg of diet: 0.08g/kg moisture, 0.06 g/kg crude protein, 0.52 g/kg ash, 0.20 g/kg calcium, 25,744.50 IU/kg Vitamin A, 1,380.00 IU/kg Vitamin D, 119.83 IU/kg Vitamin E, 21.38 mg/kg thiamine, 7.08 mg/kg riboflavin, 18.28 mg/kg pantothenic acid, 97.10 mg/kg niacin, 8.31 mg/kg pyridoxine, 1.08 mg/kg folic acid, 0.11 mg/kg biotin, 0.03 mg/kg vitamin B12.

[2]Trace mineral premix per kg of diet: 0.01 g/kg moisture, 0.22 g/kg calcium, 0.2 mg/kg sodium, 0.01 g/kg magnesium, 38.91 mg/kg iron, 11.23 mg/kg copper, 5.84 mg/kg manganese, 88.00 mg/kg zinc, 1.58 mg/kg iodine, 0.31 mg/kg selenium, 0.19 g/kg carbohydrate, and 0.01 g/kg crude fat.

[3]Dry natural antioxidant: mixed tocopherols, citric acid, rosemary extract, and soybean oil.

Table 3.2 Nutrient composition of the whole soybeans (WSB).

Item	WSB
Dry matter, g/kg, as fed	927.7
Dry matter basis	
Organic matter, g/kg	949.2
Crude protein, g/kg	385.0
Acid hydrolyzed ether extract, g/kg	209.3
Total starch, g/kg	11.6
Total dietary fiber, g/kg	198.0
Gross energy, kcal/kg	5,574

Table 3.3 Least square means and contrasts (WSB0 vs WSB100-300(T), linear (L); quadratic (Q); cubic (C) level of WSB) for nutritional composition of the experimental diets with increasing levels of WSB (WSB0, 0 g/kg, as fed; WSB100, 100 g/kg, as fed; WSB200, 200 g/kg as fed; and WSB300, 300 g/kg, as fed)

Item	WSB0	WSB100	WSB200	WSB300	SEM	WSB0 v.s. T	*L*	*Q*	*C*
Uncoated kibbles (off the dryer)									
[1]N	3	3	3	3					
Dry matter, g/kg, as fed	939.6	927.7	923.9	922.3	1.73	<0.001	<0.001	0.018	0.471
			Dry matter basis						
Organic matter, g/kg	936.7	934.5	931.6	928.3	0.62	<0.001	<0.001	0.432	0.917
Crude protein, g/kg	324.3	313.1	297.5	305.3	1.04	<0.001	<0.001	<0.001	<0.001
Acid hydrolyzed ether extract, g/kg	57.8	75.9	89.2	112.1	1.75	<0.001	<0.001	0.201	0.106
Gross energy, kcal/kg	4707.98	4733.76	4826.46	4885.00	7.035	<0.001	<0.001	0.048	0.012
Coated kibbles (off the coater)									
[1]N	3	3	3	3					
Dry matter, g/kg, as fed	942.8	929.1	927.2	926.0	1.59	<0.001	<0.001	0.005	0.157
			Dry matter basis						
Organic matter, g/kg	943.7	937.7	934.0	928.2	1.28	<0.001	<0.001	0.910	0.462
Crude protein, g/kg	303.5	294.3	284.8	298.1	1.26	<0.001	0.002	<0.001	0.004
Acid hydrolyzed ether extract, g/kg	116.6	126.5	135.3	137.1	2.66	0.007	<0.001	0.166	0.637
Gross energy, kcal/kg	4931.98	4994.66	4918.08	4913.68	24.822	0.732	0.270	0.214	0.093

[1]N = number of replicates

Table 3.4 Variable inputs of the pre-conditioner (PC) and extruder (EX) used to produce whole soybean (WSB) diets at 0, 100, 200, and 300 g/kg, as fed inclusion (WSB0, WSB100, WSB200 and WSB300), reported as average ± standard deviation.

Item	WSB0	WSB100	WSB200	WSB300
[1]N	22	24	24	20
[2]PC cylinder speed, rpm	185 ± 0	185 ± 0	185 ± 0	185 ± 0
[2]PC water, kg/h	58.9 ± 0.19	58.7 ± 0.14	58.5 ± 0.15	58.5 ± 0.20
[2]PC steam, kg/h	53.8 ± 0.53	53.1 ± 0.58	53.2 ± 0.86	53.2 ± 0.66
[3]EX screw speed, rpm	425 ± 0	425 ± 0	425 ± 0	425 ± 0

[1]N = number of replicates (data collection times during one run automatically by sensor readout) for each treatment; [2]PC = pre-conditioner; [3]EX = extruder

Table 3.5 Least squares means and contrasts (WSB0 vs WSB100-300 (T), linear (L); quadratic (Q); cubic (C) level of WSB) for processing outputs parameters used to produce whole soybean (WSB) diets at 0, 100, 200, and 300 g/kg, as fed inclusion (WSB0, WSB100, WSB200 and WSB300).

Item	WSB0	WSB100	WSB200	WSB300	SEM	WSB0 v.s.*T*	*L*	*Q*	*C*
[1]N	22	24	24	20					
[2]PC discharge temp, °C	86.77	86.46	88.10	89.19	0.252	<0.001	<0.001	0.007	0.025
[3]EX load (amps)	68.55	65.90	64.54	63.17	0.163	<0.001	<0.001	<0.001	0.069
[3]EX die temp, °C	102.95	101.58	100.72	99.58	0.117	<0.001	<0.001	0.305	0.116
[3]EX die pressure, PSI	225	300	300	200	2.671	<0.001	<0.001	<0.001	0.034
[4]SME, kJ/kg	95.6	84.0	78.1	72.1	0.717	<0.001	<0.001	<0.001	0.071
[5]TMF, kg/h	500.5	500.2	500.0	499.9	0.049	<0.001	<0.001	0.012	0.391

[1]N = number of replicates (data collection times during one run automatically by sensor readout) for each treatment; [2]PC = pre-conditioner; [3]EX = extruder; [4]SME = specific mechanical energy; [5]TMF = total mass flow.

Table 3.6 Least squares means and contrasts (WSB0 vs WSB100-300(T), linear (L); quadratic (Q); cubic (C) level of WSB) for the physical characteristics of whole soybean (WSB) diets at 0, 100, 200, and 300 g/kg, as fed inclusion (WSB0, WSB100, WSB200 and WSB300).

Item	WSB0	WSB100	WSB200	WSB300	SEM	WSB0 vs.*T*	*L*	*Q*	*C*
Off the extruder									
[1]N	3	3	3	3					
Wet bulk density, g/L	359.6	413.5	463.5	514.2	5.638	<0.001	<0.001	0.780	0.852
Kibble diameter, mm	15.33	15.28	14.33	13.66	0.167	0.002	<0.001	0.098	0.151
Kibble length, mm	5.47	5.53	5.35	5.27	0.093	0.417	0.095	0.506	0.444
Piece [2]SEI, mm^2/mm^2	2.61	2.59	2.28	2.07	0.055	0.002	<0.001	0.129	0.162
Off the dryer									
[1]N	3	3	3	3					
Dry bulk density, g/L	327.3	391.3	443.3	494.7	3.621	<0.001	<0.001	0.118	0.504
Kibble diameter, mm	15.99	14.46	13.90	12.85	0.204	<0.001	<0.001	0.273	0.143
Kibble length, mm	6.13	5.17	5.24	5.11	0.139	<0.001	0.001	0.017	0.080
Piece volume, cm^3	1.23	0.85	0.79	0.66	0.043	<0.001	<0.001	0.020	0.074
Specific length, cm/g	1.26	1.14	1.10	1.13	0.031	0.005	0.013	0.043	0.982
Piece [2]SEI, mm^2/mm^2	2.84	2.32	2.15	1.83	0.066	<0.001	<0.001	0.160	0.134
Kibble mass, g	0.49	0.45	0.48	0.45	0.008	0.017	0.064	0.408	0.011
Piece density, g/cm^3	0.39	0.54	0.60	0.68	0.026	<0.001	<0.001	0.276	0.409

[1]N = number of replicates (sample collecting times during one run) for each treatment; [2]SEI = sectional expansion index.

Table 3.7 Least squares means and contrasts (WSB0 vs WSB10-30 (T), linear (L); quadratic (Q); cubic (C) level of WSB) for the trypsin inhibitor, urease activity and phytic acid in raw and processed diets (extruded and dried) containing whole soybean (WSB) at 0, 100, 200, and 300 g/kg, as fed inclusion (WSB0, WSB100, WSB200 and WSB300).

Item	[4]WSB	WSB0	WSB100	WSB200	WSB300	SEM	WSB0 vs. *T*	*L*	*Q*	C
Raw (rations)										
[1]N	N/A	3	3	3	3					
Trypsin inhibitor ([2]TIU/g)	16,648	1,150	8,000	16,933	23,133	379.7	<0.001	<0.001	0.417	0.022
Urease (net pH increase)	2.17	0.03	0.18	1.22	2.22	0.025	<0.001	<0.001	<0.001	<0.001
Phosphorous ([3]W/W%)	0.53	0.78	0.78	0.75	0.76	0.010	0.246	0.089	0.790	0.152
Phytic acid ([3]W/W%)	1.15	1.84	1.79	1.66	1.66	0.086	0.186	0.110	0.750	0.610
Processed (kibbles)										
[1]N		3	3	3	3					
Trypsin inhibitor ([2]TIU/g)		858	2,578	3,794	5,089	136.4	<0.001	<0.001	0.158	0.369
Urease (net pH increase)		0.01	0.04	0.06	0.07	0.003	<0.001	<0.001	0.008	0.521
Phosphorous ([3]W/W%)		0.82	0.78	0.74	0.77	0.015	0.016	0.021	0.085	0.258
Phytic acid ([3]W/W%)		1.98	1.76	1.59	1.68	0.052	0.001	0.002	0.017	0.403

[1]N = number of replicates (sample collection times during one run) for each treatment; [2]TIU/g = Trypsin inhibitor unit per gram; [3]W/W% = grams per 100 g of sample, [4]The WSB column was excluded from the statistical analysis.

Chapter 4 - Apparent Total Tract Digestibility and Palatability of Extruded Diets with Graded Levels of Whole Soybeans by Dogs

4.1 Abstract

Fat has high energy density and is considered one of the primary energy sources for dogs, however, increasing fat level in dry dog food has been challenging due to the lubrication and limitation of the coating system. The objective was to determine the effect of whole soybeans (WSB) on nutrient digestibility, stool quality, and palatability by dogs. The corn gluten meal, chicken fat, and brewers rice were replaced by WSB at 10%, 20%, and 30% (WSB10, WSB20, and WSB30, respectively) in the base diet (WSB0). Twelve beagles were randomly assigned. The digestibility trial was duplicated 4 × 4 Latin square design where dogs were allowed a 9-d adaptation followed by a 5-d total fecal collection for each period. Least-square means were analyzed with a single degree of freedom contrasts and significance at $\alpha = 0.05$. Palatability was determined with a 2-bowl test by twenty beagles for 2 d with each WSB diet compared to the

WSB0. First choice preference between two diets and total food consumption were recorded. Individual intake ratios (IR) were calculated (intake of each diet/total intake) for each dog. First choice (FC) was analyzed by a Chi-square probability, and the diet consumption was compared by a Wilcoxon signed rank test and a 2-way analysis of variance. Fecal moisture, output, and defecation frequency increased linearly ($P < 0.05$) as WSB increased. Apparent total tract digestibility of dry matter, organic matter, crude protein, fat, and gross energy decreased linearly ($P < 0.05$) as dogs fed the increased level of WSB. The fresh fecal pH in dogs decreased linearly ($P < 0.05$) as WSB content increased. The acetate, propionate, and the total short-chain fatty acid concentration increased linearly ($P < 0.05$) while the total branched-chain fatty acid concentration decreased linearly ($P < 0.05$) as WSB increased. Dogs had greater ($P < 0.05$) FC for WSB diets than WSB0, but there was no difference among treatments for diet consumption and IR. In conclusion, additional thermal processing before extrusion may improve nutrient digestibility of WSB. The stool quality and palatability were not affected, and fermentation in hindgut increased by WSB by dogs.

4.2 Introduction

The U.S. pet industry had $50 billion in pet food and treats expenditures in 2021 (American Pet Products Association, 2022). As the pet food market has grown, the categories have become more differentiated to meet the specific needs of animals and their owners. For example, working dogs have higher energy requirements compared to dogs at maintenance (Wakshlag and Shmalberg, 2014). Fat has high energy density and is considered one of the primary energy sources for dogs, and thus, fat level needs to be adjusted to maintain appropriate caloric intake as activity level increases (Zoran, 2021). Glucose oxidation is the principal source of energy of energy expenditure in dogs, but fat oxidation still provides some energy and may

affect maximal energy expenditure in dogs undertaking endurance exercise (Hill et al., 2000). Interestingly, feeding diets high in polyunsaturated fats may improve olfactory ability (Angle et al., 2014) suggesting that an increase in fat level from vegetable oils may be even more beneficial for some working dogs. However, increasing fat level in dry dog food has been challenging due to the lubrication in the extrusion process and limitation in the coating system (Kim and Aldrich, 2023). Kim et al. (2022) reported that introducing intrinsic fats derived from whole soybeans (WSB) had less negative impact on processing compared to a liquid fat.

The use of vegetable proteins in animal foods has become important to address consumer concerns about the health and safety of animal protein byproducts (Willis, 2003). Soybean is the most essential oilseed crop grown in the U.S., and soybean meal (SBM) is the major source of protein for the livestock feed. In a previous report, the WSB contained 38.50 % (dry weight basis) crude protein (Kim and Aldrich, 2023) and anti-nutritional factors that decreased the bioavailability in dogs. These results can be improved with proper heating to eliminate the anti-nutritional factors (Konstance et al., 1998; Purushotham et al., 2007). The WSB also consisted of approximately 8% soy hulls (Kim et al., 2021), and the soy hulls contained 63.8 ~ 81.2% of total dietary fiber (TDF; Cole et al., 1999). Extrusion degraded the lignocellulosic structure and improved enzymatic hydrolysis of soybean hulls (Yoo et al., 2012). Thus, extrusion processing may improve the bioavailability of fibers in soybean hulls in monogastric animals (Dust et al., 2004). Further, the soluble fiber and oligosaccharides (OS) in soy may be beneficial for dogs serving as a prebiotic fiber which can be fermented and utilized as an energy source by the hindgut microbiome (Kim et al., 2023). Soy OS refer to galactosyl-sucrose raffinose and stachyose in soybeans that are non-digestible (Mussatto and Manchilha, 2007). Hernot et al. (2009) reported that the galactooligosaccharides caused the greatest production of total SCFA at

all time points during in vitro fermentation experiments in human subjects, compared to fructans and polydextrose.

There have been numerous research reports that described an increase in bioavailability of the extruded soy-based products fed to rainbow trout, broiler chicks, and pigs (Cheng and Hardy, 2003; Jahanian and Rasouli, 2016; Woodworth et al., 2001). What has not been elucidated is an optimal level of whole soybean OS for dogs. Our hypothesis was that the WSB inclusion in extruded diets would not have a negative impact on nutrient digestibility and would benefit the gut health of dogs. Therefore, the objective of this study was to determine the effects of incremental levels of WSB on the total tract apparent digestibility, stool quality, and palatability of extruded diets by dogs.

4.3 Materials and Methods

All animal procedures were approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC) under protocol #4097.

4.3.1 Experimental diets

Four diets were formulated to be nutritionally balanced for adult dogs (AAFCO, 2020a). The corn gluten meal, chicken fat, and brewers rice were replaced by WSB at 10%, 20%, and 30% (WSB10, WSB20, and WSB30, respectively) in the base diet (WSB0, control) (Table 4.1) in order to maintain the diets protein and calorie content. The experimental diets were formulated to be consistent with a premium dog food with high-protein and moderate level of fat (> 25% CP and >12% CF). Diets were formulated to have similar nutritional composition and included titanium dioxide (TiO_2; 0.4%) as an indigestible marker for determination of apparent total tract digestibility (ATTD) of dietary nutrients. As predicted by the formulation, the concentrations of minerals; calcium, phosphorous, potassium, magnesium, sodium, sulfur, manganese, copper iron,

and zinc were similar among diets and met AAFCO (2020a) nutrient profile recommendations for adult dogs at maintenance.

Raw materials were blended by a commercial mill (Fairview Mills, Seneca, KS, USA) and were ground via a hammer mill to pass through a 2-mm screen. Whole soybeans were supplied by MKC (Manhattan, KS, USA). They were cleaned using a grain cleaner and ground with a hammer mill (model 18-7-300; Schutte Buffalo, NY, USA) to pass through a 1.19-mm screen (3/64") at the Hal Ross Flour mill (Manhattan, KS, USA). Mixing of the dry raw materials and extrusion were conducted at a local extrusion pilot plant (ExtruTech Inc.; Sabetha, KS, USA) under procedures described previously by Kim and Aldrich (2023). All ingredients except the chicken fat and digest (dry dog flavor) were mixed in a ration prior to single screw extrusion. Extrudates were dried post-extrusion and applied for chicken fat and dry digest coating. While the amount of chicken fat declined with each increment of added soybeans according to the study design, the topical addition of fat was maintained > 2.0% as is typical for the minimum acceptable level for commercial pet food production.

4.3.2 Animal feeding

Dogs were housed at the Large Animal Research Center at Kansas State University (Manhattan, KS, USA). Twelve healthy adult beagles (eight neutered male and four spayed female) of similar age (6.25 ± 0.45 years) and initial body weight (BW, 12.28 ± 1.51 kg) were individually housed in metabolic pens (1.83 m × 1.20 m) equipped with an acrylic-mesh floor to allow for the separation of urine and feces. All dogs selected for this study had a body condition score ranging between 5 and 6 on a 9-point scale, with 1 being very thin, 4 to 5 being ideal, and 9 being excessively obese (Laflamme, 1997). The dogs were maintained as six dogs per room in a

temperature-controlled (23 °C) modular building with automatic light timers set to 16:8 h (light:dark) for each 24-h cycle.

The dogs were randomly assigned and fed a specific diet (WSB0: corn-wheat based diet with no WSB; WSB10: diet with 10% WSB; WSB20: diet with 20% WSB; WSB30: diet with 30% WSB). Initial dietary intake on day 0 was determined by weighing the dogs and calculating the dogs' daily metabolizable energy (ME) requirements for an average for laboratory kennel dogs (130 * BW^0.75; National Research Council, 2006). The ME of the experimental diets was calculated using the predicted equations in dog foods ((8.5 * Crude fat) + (3.5 * Crude protein) + (3.5 * Nitrogen-free extract (NFE)); National Research Council, 2006). The food intake was calculated using the dogs' daily ME relative to the predicted ME for the diets. Food allowance was controlled for each animal and feeding twice daily (at 0800 and 1700 h) in equal portions at each meal. Orts were removed and weighed after the feeding. Throughout the study, dogs were weighed weekly, and their food allowance was adjusted by 5% or 10% for the subsequent week to maintain their BW. Water was provided for *ad libitum* consumption.

4.3.3 Sample collection

The study was conducted in a replicated 4 × 4 Latin square design consisting of four periods with 9 d of adaptation to the diet followed by 5 d of total fecal collection for a total duration of 56 d. Random assignment of experimental treatments to each of the 12 dogs was carried out with the aid of a Balanced Latin Square Design Excel spreadsheet-based program (Kim and Stein, 2009). After the 9 d of adaptation, fecal samples were collected and scored on a 5-point scale following the method used in Acuff and Aldrich (2021) wherein: 1 = liquid diarrhea, 2 = very soft consistency, unformed stool; 3 = soft stool that retains shape; 4 = well-formed firm stool that does not leave residue when picked up; and 5 = very hard, dry stool. A

fecal score range of 3.5 to 4 was considered ideal. The defecation frequency was determined by counting the number of feces excretions per dog per day. After scoring, feces were collected in individual whirl-pak bags, weighed, and stored frozen at -20 °C until further analysis. During the 5-d collection period, one fresh fecal sample from each dog was immediately collected (within 15 min of excretion) and measured for pH by inserting a calibrated glass-electrode pH probe robe (FC240B, Hanna Instruments, Smithfield, RI) directly into the sample in triplicate. After measuring the pH, the fresh fecal samples were collected in three 2-mL microcentrifuge tubes and stored at -80 °C for further analysis of SCFA, BCFA (branched-chain fatty acids), and ammonia. After each collection period, bagged fecal samples were thawed at room temperature, pooled by dog, weighed, and dried in a forced air oven at 55 °C for up to 48 h until the moisture level was below 10%. This initial drying step avoided bacterial or mold growth until fecal nutrients were analyzed. The partially dried fecal samples were also weighed, and the values were used when calculating the DM (dry matter) of the fecal samples. Diet samples and partially dried fecal samples were ground using a laboratory fixed blade impact mill (Retsch, type ZM200; Haan, Germany) fitted with a 1-mm screen and stored in lidded glass jars at room temperature in preparation for chemical analysis.

4.3.4 Chemical analysis

All chemical analysis was performed in duplicate unless otherwise, specified. The WSB, experimental diets, and fecal samples were ground using a fixed blade laboratory mill (Retch, type ZM200, Haan, Germany) fitted with a 1.0-mm screen and stored in lidded glass jars in preparation. The ground WSB, experimental diets (after coating), and fecal samples were analyzed for DM, organic matter (OM), and ash according to the methods of Association of Official Analytical Chemists (AOAC, 2019; methods 934.01 and 942.05). Crude protein (CP)

content of the samples was analyzed using a nitrogen analyzer (FP928, LECO Corporation, Saint Joseph, MI) by the Dumas combustion method (AOAC 990.03). Acid hydrolyzed ether extract (AHEE) was determined by acid hydrolysis (AOAC 964.02). Gross energy (GE) was analyzed by a bomb calorimetry (Parr 6200 Calorimeter, Parr Instrument Company, Moline, IL). The titanium dioxide content in the samples was analyzed according to the colorimetric method described by Myers et al. (2004). Total starch (TS) content of the samples was determined following the standard procedure from the Total Starch Assay Kit (K-TSTA-100A, Neogen, Lansing, MI). The TDF content of the samples was measured by following the standard procedure from the Total Dietary Fiber Assay Kit (K-TDFR-200A, Neogen, Lansing, MI). The WSB, rations, and dried extrudate samples were sent to a commercial analytical laboratory (Agricultural experiment station chemical laboratories, Columbia, MO) to determine phytate, urease activity, and trypsin inhibitor activity. Phytate was analyzed according to AOAC 986.11 method. Urease activity and trypsin inhibitor activity were analyzed according to AACC international approved methods of analysis (AACC, 2006; methods 22-90 and 22-40).

Ammonia concentration in the fresh fecal samples was analyzed according to the colorimetric method described by Chaney and Marbach (1962). The fresh fecal samples kept frozen at -20 ºC for SCFA and BCFA analysis were thawed and diluted with deionized water and homogenized. The homogenized samples were centrifuged at 3,000 g for 20 min to separate the suspended solids. The 1 mL of the supernatant of the centrifuged samples was collected and 0.25 mL of 25% m-phosphoric acid was added to acidify the sample. The acidified samples were frozen at -20 ºC for at least 24 hours to complete deproteinization. The acidified samples were thawed, centrifuged at 20,000 g for 15 min, and filtered through 0.2-µm PTFE filters with a syringe.

Fecal SCFA and BCFA contents were analyzed on a gas chromatography (GC) (Erwin et al., 1961) equipped with flame ionization detector (FID) and a capillary column (BP-FATWAX UI, Agilent G3903-63008, 30 m × 0.25 mm × 0.25 µm, Agilent Technologies, Santa Clara, CA). Helium was used as a carrier gas with a flow rate of 40 cm/s, and the split ratio was 25:1 with injection volume of 0.5 µL. Hydrogen was used as the makeup gas with a flow rate of 25 mL/min. The detector and injector temperatures were set at 250 °C, and the initial oven temperature was set to 80 °C with a ramp rate of 10 °C/min to 200 °C for a total run time of 15 min. The peak area of chromatograms was determined using an integrative software (GC solution version 2.42.00, Shimadzu, Kyoto, Japan). The concentrations of SCFA (acetate, propionate, and butyrate) and BCFA (isobutyrate, isovalerate, and valerate) in the supernatant of the fecal samples were quantified by comparing the sample peak area to a standard with 10 mM of each volatile free acid (Volatile Free Acid Mix, Sigma-Aldrich, St. Louis, MO) and correcting for the fecal DM content.

4.3.5 Digestibility calculation

Two methods were utilized to estimate apparent total tract nutrient digestibility. The TFC method requires the collection of all feces excreted by the experimental animals. The marker method uses an indigestible dietary marker such as Cr_2O_3 or TiO_2 (Alvarenga et al., 2019). In the current study, apparent total tract digestibility (ATTD) of DM, OM, CP, CF, GE, and TDF was calculated according to the TFC (National Research Council, 2006) and marker methods (AAFCO, 2020b):

(1) TFC method:

$$Nutrient\ Digestibility, \% = \frac{nutrient\ consumed\ (g/d) - nutrient\ excreted\ (g/d)}{nutrient\ consumed\ (g/d)} \times 100$$

(2) Marker method:

$$Nutrient\ Digestibility, \quad \% = 1 - \frac{\%\ Nutrient\ in\ Feces\ \times\ \%\ TiO2\ in\ Food}{\%\ Nutrient\ in\ Food\ \times\ \%\ TiO2\ in\ Feces} \times\ 100$$

4.3.6 Palatability assessment

Palatability testing was conducted at a commercial research kennel (Summit Ridge Farms; Susquehanna, PA, USA) with a two-bowl test by beagle dogs (n = 20) for 2 days with each WSB containing diets compared to the control. Twenty male and female Beagles identified by ear tattoo and cage number were presented the test diets on an individual basis. Two stainless steel bowls, each containing approximately 400 grams of diet, were offered once daily for 2 days. Bowl placement was reversed daily and both bowls were presented for 30 min. If one diet was completely consumed prior to the end of the 30 min, both bowls were removed. The total quantity of the food consumption and first choice (FC) preference were recorded for each dog. Individual intake ratios (IR) were calculated by dividing the intake of each diet into the total intake for both test diets. Preference was achieved by reviewing the average intake ratios for each dog in the test and scoring one point for the diet with an intake ratio greater than or equal to 0.6667.

The kennel facility is registered with the USDA No. 23-R-0126 under the Animal Welfare Act. The kennel had a 12 h:12 h (light:dark) for each 24-h cycle and the temperature was controlled within targeted conditions range from 10 to 29.4 °C in accordance with the Animal Welfare Act. Cages and food bowls were cleaned daily and sanitized in accordance with the Animal Welfare Act.

4.3.7 Statistical analysis

The nutrient ATTD, food intake, fecal output, fecal moisture, fecal score, defecation frequency, fresh fecal pH, SCFA, BCFA, and ammonia contents from the fresh fecal samples were analyzed using the GLIMMIX procedure of SAS (version 9.4, SAS Institute, Inc., Cary,

NC). The treatment and period were the fixed main effects and dog(square) and square were random effect. Least square means of nutrient ATTD, fecal parameters, and fermentation parameters were analyzed with a single degree of freedom orthogonal contrasts. The P-values were reported for "control vs. treatments," linear, quadratic, and cubic effects of nutrient ATTD and fecal parameters by dogs fed each treatment. The results were considered significant at $P < 0.05$ and trends were considered at $0.05 \leq P < 0.10$.

FC was analyzed by a Chi-square probability, and the consumption of each diet (control vs. treatment) was compared by a Wilcoxon signed rank test and a two-way analysis of variance (ANOVA). The results were considered significant at $P < 0.05$.

4.4 Results

4.4.1 Feed types and nutrient composition

The WSB used for this study (Table 4.2) contained 7.2% moisture. On DM basis, WSB contained CP (38.5%), CF (20.9%), TDF (19.2%), ash 5.1%, low total starch (1.2%), and GE (5,574.3 Cal/g), and the anti-nutritional factors for the WSB on as-is basis were Trypsin inhibitor (> 16,000 TIU/g), urease activity (2.2 net pH increase), and phytic acid (1.2%). The WSB0 diet was within normal production parameters. The CP and OM (DM basis) were similar among diets (29.8 ± 0.65 and 93.8 ± 0.61%). Total starch content and GE tended to decrease as WSB was added to the diets but CF and TDF content tended to increase.

4.4.2 Apparent total tract digestibility

All 12 dogs remained healthy throughout the study as confirmed by veterinary staffs at the Large Animal Research Center at Kansas State University (Manhattan, KS, USA). Dogs were fed with a certain amount of food to maintain body weight without any abnormal gastro-

intestinal symptoms. This was confirmed; wherein, the BW of dogs on day 0 was 12.28 ± 1.51 kg and at day 56 was 12.82 ± 1.71 kg.

The apparent total tract digestibility of diets containing the WSB were evaluated by both, total fecal collection (TFC in Table 4.3) and by use of an indirect marker (titanium dioxide in Table 4.4). The variation (SEM) for the indirect marker method was smaller than TFC and, both data sets resulted in a linear decrease ($P < 0.05$) for ATTD of DM, OM, CF, and GE for dogs fed increasing levels of WSB diets. There was no linear decrease ($P < 0.05$) for ATTP of CP for the dogs in TFC method. There was no difference in TDF ATTD among treatments for TFC, while the TDF ATTD in WSB treatments were lower ($P < 0.05$) than the WSB0 when using indirect market method.

4.4.3 Hind-gut fermentation

There was a linear decrease ($P < 0.05$) in fresh fecal pH for dogs fed the WSB diets as WSB content increased (Table 4.5). The fecal NH_3 concentration for dogs fed WSB containing diets tended to be lower ($P = 0.054$) than those for dogs fed the WSB0. The dog fecal acetic acid, propionic acid, total SCFA, and total fatty acids increased linearly ($P < 0.05$) as WSB levels increased. On the other hand, isobutyric acid, isovaleric acid, and the total BCFA content decreased linearly ($P < 0.05$) as diet WSB inclusion level increased in the diet. For these animals, the relative proportions of propionic acid and total SCFA increased linearly ($P < 0.05$) and those of butyric acid, isobutyric acid, isovaleric acid, and total BCFA decreased linearly ($P < 0.05$) as the WSB increased in the dog diets (Table 4.6).

4.4.4 Stool quality

There was no difference among the treatments in feed intake (Table 4.7). Generally, all foods were well received and consumed by the dogs throughout the study, but the minor amounts

of orts were measured and subtracted to calculate the food intake. If food was not readily consumed by dogs within 30 min, warm water was added in order to encourage food intake.

The fecal moisture, wet fecal output, and dry fecal output were greater ($P < 0.05$) for dogs fed WSB containing diets relative to dogs fed the control diet. These variables increased in a linear fashion ($P < 0.05$) as more WSB were included in the diet. Despite this, the fecal scores were consistent among all treatments ($P > 0.05$) with scores average 3.9 ± 0.03. The defecation frequency of dogs did not differ between those fed the control diet and dogs fed the WSB containing diets ($P > 0.05$), but there was a linear increase ($P < 0.05$) in the defecation frequency as dogs were fed increasing WSB in experimental diets.

4.4.5 Canine palatability

In all cases the WSB containing diets were preferred by dogs ($P < 0.05$) relative to the control (WSB0) in FC assessment (Table 4.8). When comparing WSB10 to WSB0, 27 occasions out of 40 were chosen for WSB10 over WSB0 by dogs ($P < 0.05$). The 28 occasions out of 40 were chosen for WSB20 over WSB0 by dogs ($P < 0.05$). The 29 occasions out of 40 were chosen for WSB30 over WSB0 by dogs ($P < 0.05$). There was no difference for the food consumption and intake ratio (IR) between the WSB containing diets and the WSB0 control.

4.5 Discussion

4.5.1 Feed types and nutrient composition

The CP and CF (DM basis) of WSB used in this study (39 and 21%, respectively) were within the range reported in the literature (full-fat soya flour, 42% and 22%, Kendall and Holme, 1982; and raw soybean seed meal, 37% and 22.0%, Siulapwa and Mwambungu, 2014; raw soybean 40% and 21.7%, Vagadia et al., 2017). The experimental diets were formulated to be

isonitrogenous and isocaloric, but the internal fat and TDF included in WSB gradually increased the CF and TDF content of the diets as the WSB inclusion level increased.

4.5.2 Apparent total tract digestibility

The dogs were healthy throughout the feeding trial. Their initial BW remained constant and there were no differences in feed intake among the treatments, which is consistent with other research evaluating soy in pet food (Yamka et al., 2005). Furthermore, Menniti et al. (2014) evaluated blood parameters of healthy, adult dogs fed SBM as a replacement of up to 30% of protein provided from chicken. In their study, all blood parameters remained within normal physiological ranges. Blood chemistry was not analyzed in the current study, which is a limitation and a potential future research opportunity.

The ATTD results from TFC method can be considered by some as the gold-standard presuming that all feces are collected, and there is no loss of feces due to coprophagy. Alvarenga et al. (2019) pointed that the TFC method may lead to an overestimation of ATTD compared to the indirect marker method due to instances of loss of fecal samples by either daily pen sanitation or liquid diarrhea. However, the results from the current experiment would suggest that both TFC and indirect marker method were valid and led to the same interpretation based on statistical data analysis.

According to several studies that evaluated dietary SBM as a protein source in dog foods, CP ATTD would not be negatively affected (Yamka et al., 2003; Carciofi et al., 2009; Tortola et al., 2013; Menniti et al., 2014). However, in current study, the inclusion of WSB into the diets decreased nutrient digestibility of DM, OM, CP, CF, TDF, and GE for dogs compared to the control, WSB0. The lower nutrient digestibility in WSB-containing diets in this study could be explained by two main effects derived from WSB: fiber content from the soybean hulls and the

residual level of anti-nutritional factors from WSB. Soybean hulls contained more than 60% TDF (Dust et al., 2004) consisting of hemicellulose, cellulose, and pectin (Li et al., 2017). Cellulose is composed of strands of glucose units which are linked by 1-4 β-bonds (Knudsen, 2014). The fiber structure might have limited the enzymatic hydrolysis of the substrate, affecting nutrient bioaccessibility and the extent of macronutrient digestion and absorption (Grundy et al., 2016). On the other hand, Colonna et al. (1992) noted that dietary fibers form gels in the gastrointestinal tract and limited enzymatic hydrolysis. Moreover, Burrows et al. (1982) and Fahey et al. (1990) found that the DM digestibility and the intestinal transit time in dogs decreased with the addition of fibers. They concluded that the decreased intestinal transit time contributed to the depression of DM digestibility.

From the work of Kim and Aldrich (2023), the single extruder extrusion of the diets containing WSB did not eliminate the antinutritional factors of soybeans. Other soybean products like SBM were subjected to various processes, such as cleaning, dehulling, conditioning, flaking, boiling, or toasting, and oil extraction by either mechanical method or solvent extraction (Banaszkiewicz, 2011). The defatted soybean flakes, following oil extraction are typically subjected to processing conditions with different range of moisture, temperature and drying time to produce SBM (Thakur and Hurburgh, 2007). The WSB used in this study was raw full-fat soybean that was intact and contained high level of anti-nutritional factors prior to extrusion with the other ingredients. Felix et al. (2010) evaluated different soy protein products and reported that WSB had the highest urease and trypsin inhibitor (TI) even after the diet extrusion. Among all antinutrients present in foods, TI are of great importance since they affect protein utilization and digestion (Vagadia et al., 2017). The TI interferes with protein digestibility by forming an irreversible trypsin enzyme-trypsin inhibitor complex that declines

trypsin enzyme in the small intestine (Cabrera-Orozco et al., 2013). The negative impact due to the presence of high TI concentration in WSB-containing diets on the CP digestibility occurred in this study. Fermentable carbohydrates, such as OS may impact nutrient availability by affecting transit time and forming complexes between fibrous compounds and other nutrients (Eastwood, 1992). Smiricky et al., (2002) reported that inclusion of soy OS reduced nutrient digestibility in growing pigs, but the reduction was small.

The WSB contained approximately 21% fat (DM basis) within the seed. The internal fat derived from the WSB could have a lubricating effect during processing in the single-screw extruder. Soybean products that are high in fat content, such as soybean and micronized soybeans, reduced starch gelatinization in extruded dog foods (Felix et al., 2010). The addition of fat to the ration reduced shear inside the extruder, thereby reducing the specific mechanical energy and die temperature (Kim and Aldrich, 2023). Digestibility of starch could be affected by the degree of thermal processing since the gelatinization degree has implications on starch utilization in dogs. Less cooked starches contain a higher proportion of resistant starch and are less digestible in dogs (Alvarenga et al., 2021). The decreased degree of cooking as we increase the WSB level in diets might have reduced the DM ATTD in this study. While these differences were significant statistically, the overall level of digestibility among the diets was high (e.g., average DM ATTD 82 ± 2.6%; CP ATTD, 86 ± 1.5%; CF ATTD, 91 ± 1.4%) compared to the previous research that evaluated ATTD of SBM containing diets (e.g., DM ATTD, 80 ± 3.5%; CP ATTD, 83 ± 2.6%; CF ATTD, 88 ± 9.5%) (Vanelli et al., 2021).

4.5.3 Hind-gut fermentation

In this study, fermentable carbohydrates derived from WSB might have decreased fresh fecal pH and ammonia concentration in dogs. Moreover, the acetate, propionate, and total SCFA

concentrations increased while the total BCFA concentration decreased as the WSB inclusion level increased in the diet. Félix et al. (2013) confirmed that the fermentation of the high non-digested carbohydrates lowered the fecal pH of the dogs fed diets containing SBM. Middelbos et al. (2007) reported an increase in SCFA in beet pulp treatment compared with the control and cellulose treatments. According to Tortola et al. (2013), the SBM intake increased the fecal concentrations of propionate, acetate, and total SCFA and reduced ammonia and fecal pH, which corresponds to the current study. They concluded that soybean OS were the fermented substrate by the gut microorganisms in dogs given that the diets were similar in dietary fiber content.

Soybean OS are potential prebiotics since they are rich in galactooligosaccharides, namely stachyose, raffinose, and verbascose (Chen et al., 2010). Other reported major sugar of soybeans is sucrose, with a lower quantity of monosaccharides (Švejstil et al., 2015). According to Grieshop et al. (2003), the average stachyose, raffinose, verbascose, and sucrose contents on DM basis of 10 different soybeans was 3.8, 0.6, 0.2, and 4.8%, respectively. Similarly, Berk (1992) reported that soybeans contained 4% stachyose, 1% raffinose, and 5% sucrose. The α-galactosidic bond between sucrose and galactose that occurs in the galactooligosaccharides cannot be hydrolyzed in the small intestinal tract due to the lack of α-1,6-galactosidase (Zuo et al., 1996). Intact OS are fermented by the colon microorganisms that contain α-galactosidase (Liu, 1997) such that the non-digestible OS are indirect energy substrates and metabolic regulators (Mussatto and Mancilha, 2007). Besides the OS that is not captured from the TDF analysis, the carbohydrate in soybean consists of non-starch polysaccharides (NSP) (Choct et al., 2010). Kim et al. (2021) reported that soybean hulls contained 71.5% of total NSP. Main non-cellulosic polysaccharides from soybean hulls were mannose and xylose (Karr-Lilienthal et al., 2005).

Fermentation of dietary fibers including NSP and OS resulted in the production of SCFAs (mainly acetate, propionate, and butyrate), which reduced the pH of the intestinal lumen (Wong et al., 2006). The amounts, types, and the production rate of SCFA produced in the colon depended on the source of nondigestible carbohydrate substrate and the intestinal microflora (Mussatto and Mancilha, 2007). Acetate is partly taken-up by the liver and can be oxidized by muscle tissues throughout the body for energy in dogs (Pouteau et al., 1998). Propionate is quantitively removed from portal blood by the liver and either used as substrates for gluconeogenesis or involves in Krebs cycle at the level of succinyl coenzyme A (Wong et al., 2006). On the other hand, butyrate is extensively metabolized in the colon. Butyrate serves as the preferred energy substrate of colonocytes (Firmansyah et al., 1989; Drackley and Beaulieu, 1998). Undigested proteins are fermented and form fermentation metabolites such as BCFA, ammonia, phenolic and indolic compounds, biogenic amines, hydrogen sulfide, and nitric oxide (Gilbert et al., 2018). Nery et al. (2010) reported that the feeding high protein diets led to greater fecal concentrations of ammonia, BCFA, and valerate. Ammonia, amines, and sulfide are known to be potentially harmful in animals (MacFarlane, 1991) by shortening the colonocytes life span (Lin and Visek, 1991) and promoting tumor growth (Hughes et al., 2000). Anaerobic bacteria in the colon assimilate ammonia to form microbial protein during carbohydrate fermentation, so the ammonia concentration in the large intestine depends on the balance between amino acid deamination and bacterial protein synthesis (Hughes et al., 2000). Thus, an increase in SCFA and a decrease in pH, BCFA, and ammonia could be interpreted to positively affect intestinal health (Acuff and Aldrich, 2021).

4.5.4 Stool quality

In previous research, fecal output and score data were highly related to the TDF and non-structural carbohydrate content of soy containing diets (Clapper et al., 2001). The water-holding capacity of fiber is known to have a physiological effect on fecal bulking and shorten gut transit times, resulting in increased fecal weight and stool frequency (Roberfroid, 1993). According to Bednar et al. (2000), the soluble dietary fiber (SDF) fraction can increase the water-holding capacity of digesta resulting in greater fecal output. Muzilla et al. (1989) also reported that heat significantly increased water absorption of soy hulls which is a component of WSB. Insoluble dietary fiber (IDF) contributed to fecal bulk and promoted laxation (Dust et al., 2004). Therefore, the linear increase in fecal output, fecal moisture, and defecation frequency for dogs fed increasing levels of WSB-containing diets might be attributed to the increasing TDF content in the diets. These results are consistent with the previous studies (Yamka et al., 2003; Félix et al., 2012; Menniti et al., 2014; Corsato Alvarenga et al., 2020).

4.5.5 Canine palatability

The two-bowl forced-choice evaluation is a common method for palatability evaluation in dogs (Griffin, 2003). The IR was used to determine the preference by quantifying whether one or the other bowl was consumed in a greater proportion (Aldrich and Koppel., 2015). In the two-bowl test, the animal is allowed to smell the food before the consumption, and then the first bite from either food is recorded as FC. Thus, the FC is related to the aromatic characteristics of the food.

In the current study, dogs favored WSB containing diets over the control diet for FC, which is an indicator of aroma, but this did not result in higher consumption. Dogs are known to prefer high-fat (Hewson-Hughes et al., 2013; Hall et al., 2018) and less-fibrous foods (Koppel et

al., 2015). In the current study, WSB-containing diets had higher FC compared to WSB0. This was interesting since the amount of chicken fat applied to coat the diets were lower in WSB-containing diets than in WSB0 even though the CF content of WSB-containing diets was higher than WSB0 due to the internal fat derived from the WSB. This study found that dogs preferred higher fat containing diets from an aromatic perspective, with no preference between chicken fat and soybean oil. Similarly, Inal et al. (2020) found that sunflower oil was preferred over poultry fat by dogs. In contrast, Félix et al. (2012) reported that dogs preferred SBM-based diets over diets with poultry meal for total food consumption. It was suggested that the content of low molecular weight sugars in SBM may contribute to its greater preference by dogs (Félix et al., 2012). The WSB-containing diets had higher TDF than WSB0. According to Koppel et al. (2015), dogs preferred control diets over diets containing higher dietary fiber (sugar cane or wheat bran fibers). The higher fat content of the WSB diets might have driven higher FC in dogs, but the higher TDF content of WSB diets limited the food consumption leading to no difference in IR. This would suggest that there were no palatability issues with the increasing levels of WSB in the diet and that the size, shape, density, and texture of the product noted in the processing work (Kim and Aldrich, 2023) was not deleterious to the product acceptability by the dogs.

4.6 Conclusions

In conclusion, incremental dietary level of WSB from 0 to 30% was not harmful or deleterious to dog stool quality and palatability in this experiment. In contrast, the higher inclusion of WSB decreased the nutrient ATTD in dogs, but all levels remained high. The WSB increased the hind-gut fermentation of the diets, which can be useful to make high fiber diets for geriatric dogs or less-active adult dogs for their gut health.

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4.8 Chapter 4 Tables

Table 4.1 Diet formulas with analyzed nutrient compositions of the experimental diets with increasing levels of WSB (WSB0, 0%; WSB10, 10%; WSB20, 20%; and WSB30, 30%)

Ingredient, %	WSB0	WSB10	WSB20	WSB30
WSB	0.00	10.00	20.00	30.00
Corn	22.50	22.50	22.50	22.50
Wheat	22.50	22.50	22.50	22.50
Corn gluten meal, 60%	15.74	9.54	3.55	0.00
Rice, Brewers	8.58	6.67	4.54	0.00
Chicken meal	15.00	15.00	15.00	15.00
Beet pulp	4.00	4.00	4.00	4.00
Salt	0.50	0.50	0.50	0.50
Dicalcium phosphate	0.45	0.45	0.45	0.45
Titanium dioxide	0.40	0.40	0.40	0.40
Potassium chloride	0.25	0.25	0.25	0.25
Choline chloride, 60% dry	0.20	0.20	0.20	0.20
Fish oil	0.20	0.20	0.20	0.20
Calcium carbonate	0.15	0.15	0.15	0.15
Vitamin premix[1]	0.15	0.15	0.15	0.15
Flaxseed	0.13	0.13	0.13	0.13
Trace Mineral Premix[2]	0.10	0.10	0.10	0.10
L-Threonine 98%	0.10	0.10	0.10	0.10
Dry natural antioxidant[3]	0.04	0.04	0.04	0.04
Chicken fat (topical)	8.02	6.13	4.25	2.34
Digest - dry dog flavor (topical)	1.00	1.00	1.00	1.00
Analyzed nutrient composition				
Moisture, %	5.30	7.70	8.12	8.47
	- dry matter basis -			
Ash, %	5.64	5.91	6.42	7.02
Crude protein, %	30.48	29.79	28.92	29.98
Acid hydrolyzed ether extract, %	11.66	12.75	13.20	13.72

Total starch, %	41.38	39.82	35.98	30.83
Total dietary fiber, %	10.19	11.99	12.97	16.16
Gross energy, kcal/kg	5076.10	5001.77	4947.74	4900.92
*Metabolizable energy, kcal/kg	3506.20	3520.10	3393.50	3294.55

[1]Vitamin premix: 5.51% moisture, 4.02% crude protein, 34.5% ash, 13.4% calcium, 17,162,999 IU/kg Vitamin A, 920,000 IU/kg Vitamin D, 79,887 IU/kg Vitamin E, 14,252 mg/kg thiamine, 4,719 mg/kg riboflavin, 12,186 mg/kg pantothenic acid, 64,736 mg/kg Niacin, 5,537 mg/kg pyridoxine, 720 mg/kg Folic acid, 70 mg/kg biotin, 22 mg/kg vitamin B12.

[2]Trace mineral premix: 0.66% moisture, 21.5% calcium, 0.02% sodium, 0.57% magnesium, 38,910 mg/kg iron, 11,234 mg/kg copper, 5,842 mg/kg manganese, 88,000 mg/kg zinc, 1,584 mg/kg iodine, 310 mg/kg selenium, 19% carbohydrate, and 1% crude fat.

[3]Dry natural antioxidant: mixed tocopherols, citric acid, rosemary extract, and soybean oil.

*Calculated value: metabolizable energy = (3.5*crude protein, %) + (8.5 * acid hydrolyzed ether extract, %) + (3.5 * total starch, %).

Table 4.2 Nutritional composition of raw WSB

Item	WSB
Moisture, %	7.2
- Dry matter basis-	
Ash, %	5.1
Crude protein, %	38.5
Crude fat, %	20.9
Total starch, %	1.2
Total dietary fiber, %	19.2
Gross energy, kcal/kg	5574
Anti-nutritional factors (as-is)	
Trypsin inhibitor, [1]TIU/g	16648
Urease, net pH increase	2.17
Phytic acid, [2]W/W%	1.15

[1]TIU/g = Trypsin inhibitor unit per gram.

[2]W/W% = grams per 100 grams of sample.

Table 4.3 Least square means and contrasts (WSB0 vs. WSB10–30 [*T*]; linear [*L*]; quadratic [*Q*]) for nutrient ATTD calculated using total fecal collection method (TFC) by dogs fed diets with increasing levels (0%, 10%, 20%, and 30%) of WSB

Parameter	WSB0	WSB10	WSB20	WSB30	SEM	WSB0 v.s.*T*	*L*	*Q*
Dry matter, %	82.50	81.92	80.08	78.62	1.355	0.0246	0.0011	0.6037
			- dry matter basis -					
Organic matter, %	85.91	85.11	83.10	81.56	1.173	0.0028	<0.0001	0.6052
Crude protein, %	86.29	86.01	85.36	84.92	1.075	0.3544	0.1891	0.9204
Crude fat, %	92.10	91.85	90.58	89.98	0.540	0.0040	<0.0001	0.6358
Total dietary fiber, %	30.09	33.74	29.48	38.61	5.297	0.2722	0.1208	0.3652
Gross energy, %	86.40	85.61	83.94	82.30	1.0967	0.0061	0.0001	0.5577

Table 4.4 Least square means and contrasts (WSB0 vs. WSB10–30 [*T*]; linear [*L*]; quadratic [*Q*]) for nutrient ATTD calculated using an indirect marker method by dogs fed diets with increasing levels (0%, 10%, 20%, and 30%) of WSB

Parameter	WSB0	WSB10	WSB20	WSB30	SEM	WSB0 v.s.*T*	*L*	*Q*
Dry matter, %	85.04	82.44	79.23	79.92	0.424	<0.0001	<0.0001	<0.0001
			- dry matter basis -					
Organic matter, %	87.97	85.57	82.40	82.76	0.385	<0.0001	<0.0001	<0.0001
Crude protein, %	88.23	86.52	84.67	85.91	0.430	<0.0001	<0.0001	<0.0001
Crude fat, %	93.19	92.03	90.18	90.53	0.231	<0.0001	<0.0001	0.0011
Total dietary fiber, %	40.22	36.16	26.30	42.71	1.953	0.0003	0.6332	<0.0001
Gross energy, %	88.37	86.05	83.27	83.44	0.387	<0.0001	<0.0001	<0.0001

Table 4.5 Least square means and contrasts (WSB0 vs. WSB10–30 [*T*]; linear [*L*]; quadratic [*Q*]) for fecal pH, short-chain fatty acid (SCFA), branched-chain fatty acid (BCFA), and total fatty acids (SCFA + BCFA) production from the fresh fecal sample collected from the dogs fed diets with increasing levels (0%, 10%, 20%, and 30%) of WSB expressed in a µmol/g of feces in dry matter basis

Parameter, µmol/g of feces in dry matter basis	WSB0	WSB10	WSB20	WSB30	SEM	WSB0 v.s.*T*	*L*	*Q*
Fresh fecal pH	5.35	5.27	5.11	5.13	0.074	0.0337	0.01026	0.5063
Fecal NH_3	92.45	77.10	75.58	75.84	7.326	0.0537	0.1123	0.2751
Acetic acid	173.03	211.97	218.77	203.37	9.925	0.0001	0.0071	0.0012
Propionic acid	67.85	99.87	107.86	105.14	6.229	<0.0001	<0.0001	0.0053
Butyric acid	34.34	37.35	32.76	22.22	7.492	0.6190	0.1465	0.2788
Isobutyric acid	2.29	1.74	1.53	1.29	0.262	0.0033	0.0017	0.4609
Isovaleric acid	4.68	3.16	2.49	2.23	0.470	<0.0001	<0.0001	0.1028
Valeric acid	0.79	1.01	1.01	1.11	0.1307	0.0981	0.1109	0.6318
SCFA[1]	275.23	349.20	359.40	330.72	17.835	0.0001	0.0087	0.0010
BCFA[2]	7.76	5.92	5.02	4.63	0.744	0.0011	0.0008	0.2508
TOTAL[3]	282.99	355.11	364.42	335.35	18.100	0.0003	0.0154	0.0015

[1]SCFA, short-chain fatty acids; sum of acetic acid, propionic acid, and butyric acid.

[2]BCFA, branched-chain fatty acids; sum of isobutyric acid, isovaleric acid, valeric acid.

[3]TOTAL, total short-chain and branched-chain fatty acids; sum of SCFA and BCFA.

Table 4.6 Least square means and contrasts (WSB0 vs. WSB10–30 [*T*]; linear [*L*]; quadratic [*Q*]) for short-chain fatty acid (SCFA) and branched-chain fatty acid (BCFA) production from the fresh fecal sample collected from the dogs fed diets with increasing levels (0%, 10%, 20%, and 30%) of WSB expressed as a percentage of total fatty acids.

Parameter, %	WSB0	WSB10	WSB20	WSB30	SEM	WSB0 v.s.*T*	*L*	*Q*
Acetic acid	61.83	60.66	60.77	60.96	1.964	0.6260	0.7593	0.7117
Propionic acid	23.65	28.18	29.40	31.41	1.420	0.0004	0.0002	0.3433
Butyric acid	11.82	9.51	8.41	6.28	1.701	0.0183	0.0048	0.9462
Isobutyric acid	0.79	0.48	0.44	0.38	0.084	<0.0001	<0.0001	0.0354
Isovaleric acid	1.63	0.87	0.71	0.65	0.148	<0.0001	<0.0001	0.0015
Valeric, acid	0.27	0.29	0.28	0.33	0.040	0.5417	0.3370	0.6347
SCFA[1]	97.31	98.35	98.57	98.64	0.233	<0.0001	<0.0001	0.0070
BCFA[2]	2.69	1.65	1.43	1.36	0.233	<0.0001	<0.0001	0.0070

[1]SCFA, short-chain fatty acids; sum of acetic acid, propionic acid, and butyric acid.

[2]BCFA, branched-chain fatty acids; sum of isobutyric acid, isovaleric acid, valeric acid.

Table 4.7 Least square means and contrasts (WSB0 vs. WSB10–30 [*T*]; linear [*L*]; quadratic [*Q*]) for food intake, fecal output, fecal score, and defecation frequency of dogs fed diets containing increasing levels of WSB

Parameter	WSB0	WSB10	WSB20	WSB30	SEM	WSB0 v.s.*T*	*L*	*Q*
Intake (DM), g/d	163.95	174.61	171.62	163.30	10.385	0.4647	0.8732	0.1784
Fecal moisture, %	66.41	68.90	70.07	71.01	0.857	<0.0001	<0.0001	0.2292
Fecal output (As-is), g/d	82.02	97.11	111.23	119.11	5.369	<0.0001	<0.0001	0.4047
Fecal output (DM), g/d	27.30	30.06	32.86	34.35	1.367	0.0002	<0.0001	0.5516
Fecal score[1]	3.94	3.93	3.91	3.87	0.069	0.3959	0.1969	0.7417
Defecation frequency, times/day	1.80	1.85	1.93	2.12	0.101	0.0989	0.0106	0.4378

[1]Subjective 1 to 5 scale with 1, runny; 2, soft; 3, firm and moist; 4, firm; 5, dry and hard.

Table 4.8 Palatability assessment of diets containing WSB relative to the control (0% WSB) by dogs

Diet A vs. B	FC, n[1]	IR of diet A[2]
WSB10 v.s. WSB0	27*	0.577
WSB20 v.s. WSB0	28*	0.615
WSB30 v.s. WSB0	29*	0.632

[1]FC (first choice) number of first visits to bowl with diet A can be obtained by 40-n.

[2]IR (intake ratio) of diet A = average of intake (g) of diet A/total intake (g) of diets A + B.

**P*-value is less than 0.05.

Chapter 5 - Evaluation of Fermentability of Whole Soybeans and Soybean Oligosaccharides by a Canine In Vitro Fermentation Model

5.1 Abstract

Soybean oligosaccharides (OS) have been recognized as a prebiotic that can be fermented in the colon, resulting in short-chain fatty acid (SCFA) production that can be used as an energy source for colonocytes, supporting cell differentiation and gut health. The objective was to determine the effects of WSBOS on in vitro fermentation, using dog feces as inoculum. Treatments included total dietary fiber (TDF) residues from WSB, soybean hulls (SH), pea fiber (PF), and beet pulp (BP), as well as WSB TDF residue plus soybean OS (WSBOS) and WSB TDF residue plus raffinose, stachyose, and verbascose (WSBRSV). Fresh fecal samples were collected from dogs and maintained in anaerobic conditions until substrate inoculation. Test tubes containing fiber sources and inoculum were incubated for 4, 8, and 12 h at 39 °C. Organic matter disappearance (OMD), pH, and SCFA were measured. The WSBOS and WSBRSV had greater ($p < 0.05$) OMDs than BP. Butyrate production was greatest ($p < 0.05$) for WSBOS (294.7 µmol/g) and WSBRSV (266.1 µmol/g), followed by BP (130.3

μmol/g) and WSB (109.2 μmol/g), and lowest ($p < 0.05$) for PF (44.1 μmol/g). The production of total SCFA was greatest ($p < 0.05$) for BP and WSBOS, followed by WSB, and lowest ($p < 0.05$) for PF. In conclusion, WSB has the potential as a prebiotic demonstrating greater butyrate production than BP in a canine in vitro fermentation model due to the fermentation of both OS and fiber in WSB. Further animal feeding studies are needed to determine the appropriate amount of WSB in canine diets.

5.2 Introduction

The pet food market continues to shift toward more premiumization and use of more whole ingredients with nutrition–health-related messages. Specifically, whole soybeans (WSB) can be a nutritious ingredient for dogs because they contain 38.5% crude protein and 20.9% crude fat (DM basis) [1]. However, WSB consist of approximately 8% soybean hulls [2], which are mostly fiber (63.8 to 81.2% total dietary fiber (TDF) [3]. In addition, soybeans contain oligosaccharides (OS) in significant quantities [4], which include galactosyl-sucrose OS such as raffinose, stachyose, and verbascose [5].

Prebiotic was defined as 'a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health' in 1995 [6,7]. Galactooligosaccharides were one of the established prebiotics along with galactan, fructooligosaccharides, fructans, lactulose, oligofructose, and inulin [6,8]. The soybean OS have been recognized as prebiotics because they promote the growth of beneficial bacteria in the colon, mainly *Bifidobacterium* spp. [9].

Indigestible OS and soluble non-starch polysaccharides (NSP) in soybeans have been indicated as anti-nutritional factors in animal diets that may negatively affect diet digestibility [4,10]. There have been numerous reports that described the relationship between the poor growth performance in monogastric animals (broilers and weaning pigs) when they were fed

diets containing soybean meal that had a high fiber content, including OS and soluble NSP [11]. Moreover, due to fermentation in the large intestine, the production of gas, lactate, and short-chain fatty acids (SCFA) may result in softer feces and flatulence in dogs [12]. However, these indigestible compounds may be beneficial in the gastrointestinal tract in dogs if they are provided in the diet at an ideal dose.

Soluble fiber is known to be degraded by microbiota in the colon, resulting in SCFA production [13]; however, differences in fiber composition have a big impact on fermentation level and fermentation end-product profiles. Beet pulp (BP) is considered a standard fiber source in pet foods, with its soluble fibers providing benefit to colonic fermentation in dogs [14,15]. Pea fiber (PF) has been evaluated in an in vitro model with canine fecal inoculum, and it was intermediate in the production of SCFA compared to beet pulp and cellulose [16]. Soybean hulls (SH) were evaluated in an in vitro model with canine fecal inoculum [17], and they were intermediate in their production of total SCFA, which was lower than fructans but greater than potato starch. Prior to conducting an animal feeding trial, it is important to evaluate the fermentability of soybean OS using an in vitro model [15,16,18]. The objectives of this study were to determine the fermentation characteristics of WSB fiber residues (dietary fiber, OS, and SH) compared to traditional fiber sources, such as BP and PF, using an in vitro model with dog fecal inoculum to gain preliminary information about how WSB might function in canine diets. We hypothesized that a mixture of soybean fiber and soybean OS in ratios found in WSB would have fermentability similar to BP but greater than PF.

5.3 Materials and Methods

The dog feeding experiment was approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC) under protocol #4566.

5.3.1 Fiber Sources and Treatment Preparation

The procedures for the preparation of fiber sources were an adaptation of published methods [16]. The BP (Fairview Mills, Seneca, KS, USA), PF, and SH (Lorschters, Bern, KS, USA) fiber sources were selected to be compared with WSB residues because they have been evaluated in previous companion animal feeding studies (PF, [19]; BP, [20]). The SH and PF were ground in a laboratory-fixed blade impact mill (Retsch ZM200, Haan, Germany) to pass a 0.5 mm screen. The WSB was purchased from a local grain elevator (MKC, Manhattan, KS, USA) and ground with a hammer mill (model 18-7-300; Schutte Buffalo, NY, USA) to pass through a 1.19 mm screen. The soybean OS (WSBOS) were provided by a regional soybean processing company (Prairie AquaTech, Brookings, SD, USA). Individual galactosyl-sucrose OS (stachyose, raffinose, and verbascose) were purchased from chemical supply companies (Verbascose > 95%, Neogen, Lansing, MI, USA; Raffinose > 98%, Tokyo Chemical Industry, Tokyo, Japan; Stachyose hydrate from stachys tuberifera ≥ 90%, Chem-Impex International, Wood Dale, IL, USA).

Prior to the incubation, fiber residues from PF, BP, SH, and WSB samples were isolated using a total dietary fiber assay kit (Neogen, catalog no. K-TDFR-200A) to simulate the digestion in the small intestine of the dogs. As WSB contains significant quantities of fat, the finely ground WSB were defatted before the isolation of total dietary fiber (TDF). In this step, 100 g of ground WSB samples were placed in a 1 L beaker with a stir rod on a stirring plate under the exhaust hood. To this, 400 mL of hexane was added into the beaker and stirred for 20 min. Using a porcelain Buchner funnel and a Whatman (Marlborough, MA, USA) ashless filter paper (grade 541), the hexane mixture was filtered, and residues were dried at room temperature overnight under the hood to evaporate the hexane. The dried residues were used for TDF isolation. The isolation process followed the total dietary fiber assay protocol (Neogen, catalog no. K-TDFR-200A) with minor modifications. The PF, BP, SH, and defatted WSB were

digested with α-amylase, protease, and amyloglucosidase in bulk. A 10 g sample was mixed with 400 mL MES-TRIS buffer solution and 500 μL heat-stable α-amylase and then placed in a shaking (75 rpm) water bath at 98–100 °C for 45 min. The sample beakers were cooled to 60 °C and mixed with 1 mL protease and placed in a shaking (75 rpm) water bath set at 60 °C for 45 min. The samples were then removed from the water bath and mixed with 50 mL of 0.561 N HCl and placed on a stirring plate. The pH was adjusted to 4.5–4.7, adding either additional 5% NaOH or 5% HCl. To this, 2 mL of amyloglucosidase solution was added while stirring, and the samples were incubated in a shaking (75 rpm) water bath at 60 °C for 45 min. Then, 2250 mL of the pre-heated (60 °C) 95% ethanol was added and precipitated overnight. Using a porcelain Buchner filter and a square piece of fabric (pore size 100 microns), the precipitated solution was filtered and sequentially washed with 78% (*vol*/*vol*) ethanol, 95% (*vol*/*vol*) ethanol, and acetone. The fabric containing the residue was dried overnight in a convection oven at 105 °C. The dried residue was referred to as TDF residue from each fiber source and was used as a treatment sample (SH, PF, BP, and WSB) for the in vitro fermentation study.

The WSBOS treatment samples were prepared by mixing WSB TDF residues with soybean OS (a commercial product from Prairie AquaTech), based on the ratio of the actual TDF and total OS content of the raw WSB (TDF:OS on DM basis = 19.8:8.02) (Table 5.1). The WSBOS treatment represented the case for a dog fed WSB because WSBOS contains both the TDF residue and the OS that were filtered and lost during fiber isolation from WSB. The WSBRSV treatment samples were prepared by mixing WSB TDF residues with each oligosaccharide (raffinose, stachyose, and verbascose) in a corresponding portion of the oligosaccharides from analytical results obtained from the raw WSB (TDF/raffinose/stachyose/verbascose on a DM basis = 19.8:0.56:3.16:0.01) (Table 5.1). The difference between WSBOS and WSBRSV would largely be sucrose. The blank treatment did

not contain any substrate and was utilized to assess fermentation from substrate in the inoculum.

5.3.2 Dog Donors and Inoculum Preparation

The procedures for the preparation of the inoculum and the incubation of the fibrous substrates were an adaptation of published methods [16]. The Beagle dog donors (body weight; 7.9 ± 1.38 kg) were individually housed in the Large Animal Research Center of Kansas State University (Manhattan, KS, USA). The commercial diet (Table 5.2) was provided twice daily for each dog to maintain their body weight. Feces for the preparation of the inoculum were collected fresh within 15 min after defecation. Feces from the 3 dogs were collected immediately into plastic bags, and the air was removed from the bag to avoid exposure to aerobic conditions. The bags were placed in an insulated container that contained warm water (37 °C) to maintain temperature during transport to the lab. Samples of 33 ± 1 g of each feces was pooled together, making the total of 100 g combined fecal samples. The pooled fresh fecal samples were diluted 1:10 in an anerobic dilution solution (Table 5.3) and purged with CO_2. The solution was blended well until most of the fecal lumps were dispersed. The solution was then filtered through 4 layers of cheese cloth under purging CO_2. The filtered solution was used as the inoculum for the fermentations.

5.3.3 Canine In Vitro Microbial Fermentation

The treatment samples were subjected to in vitro microbial fermentation, as described by [16], with some modifications. Briefly, 0.3 ± 0.0001 g of the treatment samples were weighed in triplicate in 50 mL conical centrifuge tubes for each one of the 3 time points (4, 8, 12 h). In addition to the tubes with the fiber samples, tubes without any fiber samples for each time point in triplicate were used as blanks. To each tube, 26 mL of media solution was added. Next, each tube was flushed with CO_2 and closed with a rubber stopper equipped with a 1-way

Bunsen valve. Tubes were then placed in the refrigerator overnight to allow substrates to hydrate. On the following day, the samples were placed in a shaking (60 rpm) water bath at 39 °C for 45 min prior to inoculation.

Tubes were inoculated with 4 mL of the prepared inoculum using a repeater pipette, starting with tubes from time 12, 8, and, lastly, 4 h. After inoculation, tubes were flushed with CO_2, closed with a rubber stopper equipped with a 1-way Bunsen valve, and incubated in a shaking (30 rpm) water bath at 39 °C for the predetermined time points. The tubes were vortexed every 2 h. At each incubation timepoint, a 1 mL subsample from each tube was transferred to a 2 mL microcentrifuge tubes and mixed with 0.25 mL of 25% (*wt*/*vol*) m-phosphoric acid for deproteinization. The microcentrifuge tubes were frozen at −20 °C until SCFA analysis. The pH of the remaining solutions in the 50 mL conical centrifuge tubes were measured by inserting a calibrated glass-electrode pH probe (FC240B, Hanna Instruments, Smithfield, RI, USA) directly into the sample. All the remaining solution in the 50 mL conical centrifuge tube were transferred to a 400 mL beaker, mixed with 112 mL of 95% ethanol, and allowed to precipitate overnight at room temperature.

On the following day, the solutions were filtered using pre-weighed dried Whatman (Marlborough, MA, USA) ashless filter paper (grade 541) and a porcelain Buchner funnel with a vacuum pump. The filtered residues were rinsed twice with 10 mL of 95% ethanol and twice with 10 mL of acetone. Next, the filter paper containing the residues were put in a 50 mL beaker and dried in a convection oven overnight at 105 °C. The dry weights of the filter and residue were recorded the following day. After samples were weighed for DM, the 50 mL beakers containing the filter and residue were placed in the muffle furnace at 450 °C overnight and weighed on the next day for ash corrections.

Nutrient compositions were variable across the fiber sources and the TDF residues that were used as substrates in the study (Table 5.4). The TDF content of the PF was the highest

(72.9%), followed by SH (67.9%), BP (61.1%), and WSB (21.5%) on a dry matter basis. The IDF content of the PF was the highest (68.0%), following by SH (58.3%), BP (36.5%), and WSB (19.4%) on a dry matter basis. The WSB contained more CP (38.5%) on a DM basis than the other fiber sources, such as SH (17.0%), BP (15.2%), and PF (14.0%). The CP content of defatted WSB TDF residue was the highest (38.0%) and that of PF was the lowest (8.4%).

5.3.4 Determination of Organic Matter Disappearance (OMD) and Chemical Analysis

The OMD (%) were calculated as follows:

$$\text{OMD}\ (\%) = \left(1 - \frac{\text{organic matter residue(g)} - \text{organic matter blank(g)}}{\text{initial organic matter (g)}}\right) \times 100\% \quad 1)$$

wherein organic matter (OM) residue is OM in the sample after the incubation and filtration in g, OM blank is OM in the blank after incubation and filtration in g, and Initial OM is the initial OM in the sample prior to incubation in g.

All chemical analyses were performed in duplicate unless otherwise specified. The fiber samples were ground using a fixed blade laboratory mill (Retch, type ZM200, Haan, Germany), fitted with a 1.0 mm screen, and stored in lidded glass jars in preparation for chemical analysis. The ground WSB, defatted WSB, SH, BP, PF, and TDF residues were analyzed for DM, OM, and ash (AOAC methods 934.01 and 942.05). Crude protein (CP) content of the samples was determined by the Dumas combustion method (AOAC 990.03), using a nitrogen analyzer (FP928, LECO Corporation, Saint Joseph, MI, USA). The insoluble dietary fiber (IDF) and TDF content of the samples were measured following the standard procedure from the Total Dietary Fiber Assay Kit (K-TDFA-200A, Neogen). The soluble dietary fiber (SDF) was calculated subtracting IDF concentration from the TDF concentration. The WSB sample was

analyzed by the Agricultural Experiment Station Chemical Laboratories (Columbia, MO, USA) for oligosaccharides (sucrose, stachyose, raffinose, and verbascose), as described by [21].

For SCFA analysis, samples were thawed and centrifuged at 20,000 g for 15 min. The supernatant was collected and filtered through a 0.2 µm PTFE syringe filter. The SCFA contents from the filtered samples were analyzed by gas–liquid chromatography [22], using a capillary column (BP-FATWAX UI, Agilent G3903-63008, 30 m × 0.25 mm × 0.25 µm; Agilent Technologies, Santa Clara, CA, USA). The system was equipped using helium as a carrier gas, with a flow rate of 40 cm/s, utilizing a 15:1 split ratio injector, with an injection size of 0.5 µL. A flame ionization detector was configured with hydrogen as the makeup gas with a flow rate of 25 mL/min. The detector and inlet temperatures were set at 250 °C, and the initial oven temperature was set to 80 °C with a ramp rate of 10 °C/min to 200 °C for a total run time of 15 min. The peak area of chromatograms was determined using integrative software (GC solution version 2.42.00, Shimadzu, Kyoto, Japan). The concentrations of SCFA in the supernatant of the fermented samples were quantified by comparing the sample peak area to 10 mm standards (Volatile Free Acid Mix, Sigma-Aldrich, St. Louis, MO, USA). Production of SCFA was calculated per unit of substrate DM. The average SCFA concentrations from the blank tubes were subtracted to correct any artifacts that the inoculum might have had.

5.3.5 Statistical Analysis

The experiment had 7 treatments (including the blank as a treatment) × 3 timepoints × 3 replicates. It was performed with a completely randomized design, with 50 mL conical centrifuge tubes as experimental units. The OMD, pH, and SCFA data were subjected to ANOVA for a completely randomized design with factorial arrangement of the substrate and time factors using the general linear model procedure of SAS (v. 9.4; SAS Inst. Inc., Cary, NC, USA). Differences of least square means were assessed using Tukey's post hoc test for multiple comparisons. Results were considered significant at $p < 0.05$.

5.4 Results

5.4.1 OMD and pH

For the OMD and pH, the 12 h time point, which is the maximum time for the fermentation in this trial, will be discussed unless otherwise specified (Table 5.5). The BP was regarded as the reference because it is a prominent fiber source used in commercial dog food, and its fermentability by dogs is well understood [16]. Compared to BP (41.0%), WSBOS (60.2%) had a higher OMD throughout all the time points ($p < 0.05$). The WSBRSV treatment (43.6%) had a less OMD than WSBOS (60.2%) throughout all the time points ($p < 0.05$). The WSB had a lower OMD ($p < 0.05$) than BP; however, WSB (37.6%) had a higher OMD than SH (20.4%) and PF (18.6%). The pH was affected by the different fiber sources in the current study. The BP and WSBRSV had the lowest pH among the treatments ($p < 0.05$), and the pH for the PF (7.05), WSB (6.90), SH (7.07), and WSBOS (7.01) did not differ and were lower than the blank pH (7.50).

5.4.2 Short-Chain Fatty Acids

The descriptions for SCFA production presented here are based on the 12 h time point unless otherwise specified. Acetate production was greatest for BP, followed by WSBOS and WSBRSV, and lowest for PF and SH ($p < 0.05$; Table 5.6). Propionate production was greatest ($p < 0.05$) for BP, WSBRSV, and WSBOS, followed by WSB, PF, and SH. However, butyrate production was greatest ($p < 0.05$) for WSBOS (294.7 μmol/g) and WSBRSV (266.1 μmol/g), followed by BP (130.3 μmol/g) and WSB (109.2 μmol/g), and lowest ($p < 0.05$) for PF (44.1 μmol/g). The production of total SCFA was greatest ($p < 0.05$) for BP and WSBOS, followed by WSB, and lowest ($p < 0.05$) for PF.

5.5 Discussion

5.5.1 Nutritional Compositions

Sucrose, raffinose, and stachyose are major soluble sugars in soybean seeds [5]. Our WSB had similar TDF, sucrose, raffinose, and stachyose values to those previously reported by [23]. During the isolation of fiber from WSB, OS should not have been recovered in the TDF residue. Either soy OS or combinations of raffinose, stachyose, and verbascose were added to replace the loss of OS in the treatment preparations for WSBOS and WSBRSV. The TDF, IDF, and SDF contents of the PF, SH, and BP were also similar to the previous published data [3,16,20]. Dietary fiber and raffinose family oligosaccharides resisted hydrolysis by endogenous enzymes in the small intestine but may have been fermented by microbes in the colon to CO_2, H_2, ammonia, SCFA, and lactate. The source and solubility of the fiber determined the fermentation characteristics of the intestinal microbiota [24,25].

5.5.2 OMD and pH

Even though WSB contained less SDF than the SH and PF, the TDF residues of the defatted WSB contained higher CP, and this might have contributed to the higher OMD. The SH OMD reached its maximal level by 4 h. The major constituents of the total dietary fiber in SH were cellulose (30–50%), hemicellulose (15–25%), and pectin (6–15%) [26]. The high IDF content and the physical structure of SH likely contributed to impede anaerobic microorganisms to extend fermentation past 4 h. The fact that sucrose was present in WSBOS and not in WSBRSV might have partially contributed to the higher OMDs for the WSBOS than the WSBRSV. However, as the OS would not be recovered in the fermentation residues due to a small molecular size, even if they were not fermented, the OMDs of the WSBOS and WSBRSV were not necessarily reflective of the extent of fermentation.

The production of the SCFA via fermentation of carbohydrates by the gut bacteria reduces gastrointestinal luminal pH, which directly limits pathogen growth [27]. In an in vitro fermentation study using fecal samples from growing pigs [25], total gas production increased, and the pH of the fermentation substrate decreased as the SDF ratio increased compared to the IDF. Our results were similar to the research of [25] because the BP had the highest amount of SDF and the highest SDF to IDF ratio among the fibers, and this led to the lowest pH. However, the range of the pH difference was small. It is difficult to determine fermentability indirectly by pH measurement because substantial amounts of buffer solutions were added in the in vitro fermentation procedure, and the substrates can provide buffering as well. We measured the SCFA concentrations as a more definitive measure of fermentation.

5.5.3 Short-Chain Fatty Acids

Some of the health benefits produced by dietary fibers are the production of fermentative end products and changes in the gastrointestinal microbiota [27]. The main bacterial fermentative end products are SCFA and the gases H_2 and CO_2; SCFA are an important indicator of fermentation in the colon [28]. The fermented end product profile depends on the substrate source and the microbial ecology in the colon [29]. According to previous work, pectin yields high acetate [30], gum yields high propionate [31], and resistant starch, lactose, and soybean oligosaccharides yield high butyrate concentrations after microbial fermentation with fecal inoculum [32]. The higher ratio of SDF to IDF in the dietary fibers increased in the concentrations of lactic acid, formic acid, and acetic acid, whereas the concentrations of propionic acid and butyric acid were greater in the low SDF ratio group in an in vitro fermentation experiment using pig fresh fecal inoculum [25]. The SCFA are used as an energy source for colonocytes and enterocytes and influence gastrointestinal epithelial cell integrity [28]. Acetate is absorbed and transported by the portal vein and used as a fuel for tissues throughout the body. Propionate is either taken up by the liver and converted to glucose [33]

or locally utilized [34]. Butyrate is the major fuel source for colonocytes [29], increases colonocyte proliferation [35], and increases the mucin secretion in the large intestine [36]. Moreover, butyrate also influences various cellular functions affecting colonic health, such as anticarcinogenic and anti-inflammatory pathways, affects the intestinal barrier, and decreases oxidative stress [37]. Wächtershäuser and Stein (2000) [38] suggested that increasing luminal butyrate concentrations may be an appropriate means to ameliorate symptoms of inflammatory bowel diseases.

The soybean cell wall contains pectinic acid polysaccharides that contain uronic acids and neutral polysaccharides [39]. Yamaguchi et al. (1996) [40] found that pectic polysaccharides in soybeans had a similar molecular weight and galacturonan structure to that of fruit pectin. Galactose and arabinose were the main components in each of the polysaccharides. The WSB, WSBRSV, and WSBOS, which contained this pectinic acid group, had higher acetate and butyrate productions than the PF in this study. Swanson et al. (2001) [13] also found that citrus pectin produced higher amounts of acetate, butyrate, and total SCFA than pea hulls. The primary oligosaccharides found in the soybeans were galactooligosaccharides. Hernot et al. (2009) [30] reported that the galactooligosaccharides produced large quantities of SCFA, particularly butyrate, in an in vitro fermentation system. As we added soybean oligosaccharides to the WSB TDF residues, the butyrate and total SCFA production for WSBRSV and WSBOS was higher than for WSB. This was expected.

The colonic microflora might have degraded the NSP that remained in the WSB, SH TDF residues, and synthesized SCFA. Bakker et al. (1998) [41] found that the soybean hulls had more extensively fermented NSP than cellulose, yielding a higher amount of acetate, propionate, and butyrate in pigs. For our butyrate productions, WSBRSV had 144% the production of WSB, whereas WSBOS had 170% the production of WSB. These findings provided evidence that soybean galactooligosaccharides are fermented in the colon of dogs and

yield a substantial amount of butyrate compared to acetate or propionate. Lan et al. (2007) [32] reported that stachyose and raffinose produced higher butyrate contents than soybean meal oligosaccharides when inoculated with the caecal contents from broilers for an in vitro fermentation model. The OS may have more potential than the soluble fibers in WSB to serve as substrate for butyrate production. The inclusion of WSB in diets will provide both WSB TDF and OS.

Hore and Messer (1968) [42] found that sucrase was present in the small intestine of dogs. However, sucrase levels are low in dogs throughout their lives [43,44]. Buddington et al. (2003) [45] reported that the activities of sucrase increased after birth in Beagle dogs. Kienzle (1988) [46] found that sucrase activity was higher in adult dogs than puppies if the diet contained soy, lactose, and sucrose. However, the sucrase activity was similar between puppies and adult dogs if they were fed carbohydrate-free diets [46]. Therefore, sucrose may escape digestion and be fermented in the large intestine as a fermentable carbohydrate. Thus, the treatments with OS, WSBRSV, and WSBOS represented canine diets containing WSB depending on the dogs' sucrase activity levels in their small intestine. The WSBRSV and WSBOS resulted in more butyrate production than the BP, indicating that feeding WSB might have a beneficial impact on colonic health in dogs.

Isobutyrate and isovalerate are produced from fermentation of amino acids rather than carbohydrates [16,47]. According to [48], the fermentation of undigested protein yielded ammonia, valerate, and branched-chain fatty acids (isobutyrate and isovalerate) in dog feces. Panasevich et al. (2015) [49] observed no changes in markers of protein fermentation such as fecal branched-chain fatty acids with increasing soluble corn fiber (higher total dietary fiber) supplementation. These branched-chain fatty acids were generated when energy was limited in the large intestine [35]. According to [50], the absence of carbohydrates and the presence of undigested protein available in the hindgut could favor increased proteolytic activity by a

greater number of bacteria. Detweiler et al. (2019) [51] found that no fiber treatment had significantly greater branched-chain fatty acids in dog feces compared to the addition of fiber treatments. Middelbos et al. (2007) [35] suggested that the rapid fermentation of fructooligosaccharides in the proximal colon in dogs might have resulted in the limited energy environment in the distal colon, leading to an increased catabolism of amino acids. Propst et al. (2003) [52] reported higher ammonia, isovalerate, and total biogenic amines in dog feces when the dogs received dietary fructans.

In our study, WSBOS had the highest ($p < 0.05$) isobutyrate and isovalerate concentrations. The valerate concentration was the highest ($p < 0.05$) for both WSBOS and WSBRSV. Considering the treatments were inoculated with the same population of anerobic bacteria, the reason for the highest branched-chain fatty acids in WSBOS could be explained by the rapid fermentation of the OS. The butyrate concentration of WSBOS seemed to be reach the maximum at an 8 h timepoint, showing a more rapid fermentation rate than the other treatments. Middlebos et al. (2007) [35] reported that OS are highly fermentable compared with fiber and are rapidly consumed once they enter the colon. Especially, valerate concentrations increased between 8 and 12 h of fermentation more so than other SCFA. Specific bacteria such as *Megasphaera elsdenii* are known to produce valerate along with acetate, propionate, and butyrate in pig intestines [53]. The *Megasphaera elsdenii* was in the Beagle dogs' fecal microflora [54], and these bacteria might have more actively produced valerate in late timepoints in the current study.

The PF had low concentrations of butyrate, whereas SH had butyrate concentrations similar to WSB and BP. Legume hulls contain large quantities of xylan as hemicellulose polymers [39], which were identified as part of IDF and NSP. The variation in degradability of the NSP was very large due to the different degrees of cell wall lignification, particle size, and retention time in the gut [41]. For a good fermentation of NSP in the colon, an adequate amount

of nitrogen is required to feed colonic bacteria. In vivo, adequate levels of nitrogen are generally provided by residual undigested protein escaping the small intestine, endogenous nitrogen in mucus and epithelial cells, and urea recycled into the gastrointestinal tract [55]. In our in vitro system, an adequate amount of nitrogen was provided by yeast extract in the media solution. The PF TDF residues contained the least amount of CP, which might explain the lowest branched chain fatty acids concentrations. Lignin and crystallinity of cellulose in PF might have contributed to limiting the rate and extent of the microbial fermentation. To increase the fermentability of legume hulls, heat pretreatment or fiber-degrading multi-enzyme supplementation has been used in pigs [2].

According to their chemical composition and fermentative end-product concentrations, WSB can potentially be used as prebiotic ingredients based on two assumptions. Firstly, WSB contained high amount of TDF and OS that were indigestible by mammalian digestive enzymes but were fermented in the colon by the microbiome. Secondly, the WSBOS treatment, which represents the biological situation of dogs fed WSB if small intestinal digestion of sucrose is low, showed more than twice the butyrate concentrations of the BP. Butyrate is oxidized by the intestinal mucosa and serves as the preferred energy substrate of colonocytes [56,57]. Moreover, the fermentation of nondigestible carbohydrates can affect the host by stimulating the growth and activity of beneficial bacterial concentrations (i.e., *lactobacilli* and *bifidobacteria*) and decrease potentially harmful bacteria (i.e., *Escherichia coli* and *enterobacteria*) in the gut [39]. Microbiota changes were not analyzed in the current study, which is a potential future research opportunity. However, further animal feeding studies are needed to determine the appropriate dose of WSB in dogs that have minimal anti-nutritional effects and flatulence induced by OS.

5.6 Conclusions

This work demonstrated that WSB has the potential as a prebiotic, yielding more butyrate production than BP in a canine in vitro fermentation model due to both fiber and highly fermentable OS. Further animal feeding studies are needed to determine the appropriate dose of WSB in dogs with measurements of canine health and microbial populations in the gut. On the other hand, PF was poorly fermented, having a high portion of IDF. This ingredient could be included in weight control diets anticipating the larger effect of IDF than gut health in dogs.

5.7 References

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5.8 Chapter 5 Tables

Table 5.1 Oligosaccharide concentrations in whole soybeans

Oligosaccharide	% of Dry Matter
Sucrose	4.28
Raffinose	0.56
Stachyose	3.16
Verbascose	0.01

Table 5.2 Analyzed nutrient composition of the commercial diet [1] fed to dogs during and before collection of fresh feces.

Nutrient	Diet
Dry matter, %	91.4
Ash, % of dry matter	8.0
Crude protein, % of dry matter	31.2
Acid hydrolyzed ether extract, % of dry matter	15.2
Total dietary fiber, % of dry matter	14.0

[1] Ingredients: ground corn, chicken meal, corn gluten meal, rice flour, porcine meat and bone meal, dried plain beet pulp, poultry fat preserved with BHA, porcine animal fat preserved with BHA and citric acid, brewers dried yeast, hydrolyzed poultry by-products aggregate, spray dried animal blood cells, dried egg product, dried whey, L-lysine, salt, dicalcium phosphate, soybean oil, natural flavor, potassium chloride, calcium carbonate, choline chloride, pyridoxine hydrochloride, DL-methionine, menadione dimethyl pyrimidinol bisulfite (source of vitamin K), cholecalciferol (form of vitamin D3), lecithin, biotin, vitamin A acetate, DL-alpha tocopheryl acetate (form of vitamin E), ferrous sulfate, inositol, preserved with mixed tocopherols, zinc oxide, calcium pantothenate, folic acid, thiamine mononitrate, calcium iodate, ethoxyquin (a preservative), riboflavin supplement, nicotinic acid, manganous oxide, vitamin B-12 supplement, copper sulfate, cobalt carbonate.

Table 5.3 Composition of inoculation medium and anaerobic dilution solutions.

Solution	Medium	Anerobic Dilution
Solution A [1], mL	330.0	37.50
Solution B [2], mL	330.0	37.50
Mineral solution [3], mL	10.0	-
Vitamin solution [4], mL	10.0	-
Folate-biotin solution [5], mL	5.0	-
Riboflavin solution [6], mL	5.0	-
Hemin solution [7], mL	2.5	-
Resazurin solution [8], mL	1.0	1.00
Water, mL	296.0	854.00
Yeast extract, g	0.5	-
Trypticase, g	0.5	-
Na_2CO_3, g	4.0	6.37
Cysteine hydrochloride, g	0.5	0.50

[1] Solution A—5.4 g sodium chloride, 5.4 g ammonium sulfate, 2.7 g potassium phosphate monobasic anhydrous, 0.18 g calcium chloride dihydrate, 0.12 g magnesium chloride hexahydrate, 0.06 g manganese chloride tetrahydrate, 0.06 g cobalt chloride hexahydrate, to 1 L with distilled water. [2] Solution B—2.7 g potassium phosphate dibasic anhydrous to 1 L with distilled water. [3] Mineral solution—500 mg of ethylenediaminetetraacetic acid, 200 mg iron (II) sulfate heptahydrate, 30 mg m-phosphoric acid, 20 mg cobalt chloride hexahydrate, 10 mg zinc sulfate heptahydrate, 3 mg manganese chloride tetrahydrate, 3 mg sodium molybdate dihydrate, 2 mg nickel (II) chloride hexahydrate, 1 mg copper (II) chloride dihydrate, to 1 L with distilled water. [4] Vitamin solution—Weigh 100 mg thiamin hydrochloride, 100 mg pantothenic acid, 100 mg niacin, 100 mg pyridoxine hydrochloride, 10 mg ammonium carbonate, 5 mg 4-aminobenzoic acid, 0.25 mg vitamin B-12, to 1 L with distilled water. Added to the medium by filter sterilization after other reagents were sterilized in autoclave. [5] Folate-biotin solution—100 mg ammonium carbonate, 10 mg folic acid, 2 mg biotin, to 1 L with distilled water. [6] Riboflavin solution—130 mg HEPES, 1 mg riboflavin, to 1 L with distilled water. [7] Hemin solution—50 mg hemin, 40 mg sodium hydroxide, to 100 mL with distilled water. [8] Resazurin solution—100 mg resazurin to 100 mL with distilled water.

Table 5.4 Nutritional composition of fiber sources (WSB, whole soybean; DWSB, defatted whole soybean; SH, soy hull; BP, beet pulp; PF, pea fiber) and the total dietary fiber (TDF) residues isolated from the fiber sources and used as substrates.

Item	WSB	DWSB	SH	BP	PF
Fiber sources					
Dry matter, %	92.5	91.9	98.0	91.9	92.4
	-dry matter basis-				
Organic matter, %	94.9	93.7	95.1	94.6	96.9
Crude protein, %	38.5	47.8	17.0	15.2	14.0
Total dietary fiber, %	21.5 *	25.8	67.9	61.1	72.9
Insoluble dietary fiber, %	19.4 *	23.3	58.3	36.5	68.0
Soluble dietary fiber [1], %	2.1 *	2.5	9.6	24.6	4.9
TDF residues					
Dry matter, %	n.d. [2]	90.5	90.9	89.3	89.7
	-dry matter basis-				
Organic matter, %	n.d.	94.9	97.0	94.0	97.7
Crude protein, %	n.d.	38.0	12.6	12.9	8.4

[1] Calculated as total dietary fiber—insoluble fiber.
[2] n.d. means not determined.
* Calculated values using analysis results for defatted WSB samples.

Table 5.5 Least square means of organic matter disappearance (OMD, %) and pH of fermented fiber sources inoculated with dog feces for 4, 8, and 12 h (PF, pea fiber; WSB, whole soybeans; BP, beet pulp; SH, soy hulls; WSBRSV, WSB plus raffinose, stachyose, and verbascose; WSBOS, WSB plus soy oligosaccharides).

Incubation Time, h	Blank	PF	BP	SH	WSB	WSBRSV	WSBOS	SEM [1]	*p*-Value
OMD, %									
4 h	.	15.8 [d]	39.3 [b]	19.6 [d]	28.8 [c]	37.2 [b]	58.3 [a]	1.70	<0.0001
8 h	.	19.3 [c]	41.2 [b]	18.5 [c]	34.9 [b]	38.6 [b]	55.2 [a]	1.34	<0.0001
12 h	.	18.6 [d]	41.0 [b]	20.5 [d]	37.6 [c]	43.6 [b]	60.2 [a]	0.60	<0.0001
pH									
4 h	7.05 [a]	6.94 [ab]	6.49 [d]	6.82 [bc]	6.79 [bc]	6.64 [cd]	6.71 [cd]	0.046	<0.0001
8 h	7.05 [a]	6.99 [a]	6.60 [b]	6.95 [ab]	6.96 [ab]	6.76 [ab]	6.92 [ab]	0.080	0.0193
12 h	7.50 [a]	7.05 [b]	6.66 [d]	7.07 [b]	6.90 [bc]	6.74 [cd]	7.01 [b]	0.041	<0.0001

[1] SEM = standard error of the mean.

[abcd] Means with different lowercase letters in the same row are significantly different at $p < 0.05$.

Table 5.6 Least square means of short-chain fatty acid (SCFA) production from fermented fiber sources (PF, pea fiber; WSB, whole soybeans; BP, beet pulp; SH, soy hulls; WSBRSV, WSB plus raffinose, stachyose, and verbascose; WSBOS, WSB plus soy oligosaccharides) inoculated with dog feces for 4, 8, and 12 h, expressed as mmol/g of substrate dry matter.

Fermentation Time, h	PF	BP	SH	WSB	WSBRSV	WSBOS	SEM [1]	*p*-value
Acetate, µmol/g of substrate								
4	458 ^{d}	1876 ^{a}	794 ^{c}	671 ^{cd}	1232 ^{b}	1411 ^{b}	54.5	<0.0001
8	616 ^{e}	2172 ^{a}	873 ^{de}	1009 ^{d}	1476 ^{c}	1858 ^{b}	60.4	<0.0001
12	832 ^{e}	2844 ^{a}	1060 ^{de}	1415 ^{cd}	1817 ^{bc}	2123 ^{b}	86.2	<0.0001
Propionate, µmol/g of substrate								
4	176 ^{d}	482 ^{bc}	314 ^{cd}	243 ^{d}	570 ^{ab}	698 ^{a}	37.1	<0.0001
8	228 ^{d}	606 ^{b}	326 ^{c}	356 ^{c}	657 ^{b}	923 ^{a}	18.1	<0.0001
12	296 ^{b}	923 ^{a}	399 ^{b}	468 ^{b}	835 ^{a}	992 ^{a}	45.6	<0.0001
Butyrate, µmol/g of substrate								
4	27 ^{b}	66 ^{b}	64 ^{b}	77 ^{b}	205 ^{a}	249 ^{a}	15.2	<0.0001
8	32 ^{e}	78 ^{d}	50 ^{e}	105 ^{c}	220 ^{b}	308 ^{a}	5.4	<0.0001
12	44 ^{c}	130 ^{b}	63 ^{bc}	109 ^{bc}	266 ^{a}	295 ^{a}	16.6	<0.0001
Isobutyrate, µmol/g of substrate								
4	2.8 ^{c}	4.0 ^{bc}	11.9 ^{bc}	10.7 ^{bc}	15.4 ^{ab}	25.0 ^{a}	2.41	0.0003
8	4.4 ^{c}	4.1 ^{c}	8.0 ^{c}	14.6 ^{b}	18.0 ^{b}	35.9 ^{a}	1.38	<0.0001
12	5.7 ^{c}	12.7 ^{bc}	11.5 ^{bc}	14.2 ^{bc}	19.0 ^{b}	33.8 ^{a}	2.31	<0.0001
Isovalerate, µmol/g of substrate								
4	1.9 ^{c}	4.0 ^{c}	14.3 ^{bc}	13.0 ^{bc}	20.8 ^{ab}	31.5 ^{a}	3.03	0.0002
8	2.9 ^{c}	2.4 ^{c}	8.5 ^{c}	18.7 ^{b}	23.0 ^{b}	42.2 ^{a}	1.65	<0.0001
12	4.8 ^{d}	10.3 ^{cd}	12.2 ^{bcd}	19.3 ^{bc}	24.4 ^{b}	40.5 ^{a}	2.96	<0.0001
Valerate, µmol/g of substrate								
4	1.5 ^{c}	3.4 ^{bc}	3.5 ^{bc}	2.5 ^{c}	7.2 ^{ab}	9.5 ^{a}	0.93	0.0004
8	7.9 ^{c}	7.6 ^{c}	9.3 ^{c}	13.3 ^{c}	22.8 ^{b}	46.6 ^{a}	1.38	<0.0001
12	14.3 ^{d}	27.4 ^{cd}	18.8 ^{d}	45.7 ^{bc}	63.3 ^{ab}	79.0 ^{a}	4.32	<0.0001
Total SCFA, µmol/g of substrate								
4	667 ^{c}	2435 ^{a}	1201 ^{b}	1017 ^{bc}	2050 ^{a}	2424 ^{a}	111.4	<0.0001
8	891 ^{d}	2869 ^{a}	1274 ^{cd}	1516 ^{c}	2417 ^{b}	3214 ^{a}	81.4	<0.0001
12	1196 ^{d}	3948 ^{a}	1565 ^{cd}	2071 ^{c}	3025 ^{b}	3563 ^{ab}	155.2	<0.0001

[1] SEM = standard error of the mean.

^{abcde} Means with different lowercase letters in the same row are significantly different at $p < 0.05$.

Chapter 6 – Descriptive sensory analysis and consumer acceptance of extruded dog diets with graded levels of whole soybeans

6.1 Abstract

Whole soybeans have an excellent potential to be a nutritious ingredient in dog foods. Although palatability of soybeans in dog food has been studied, understanding pet food preferences can be challenging because dogs cannot verbally articulate their preferences. Additionally, there is limited research regarding consumer acceptance of soybean-containing dog foods even though there is a consumer bias against soy products in pet diets. The objectives of this study were to determine the effect of incremental levels of whole soybeans (WSB) in dry dog foods on descriptive sensory properties and consumer acceptance. Experimental diets were extruded with 10%, 20%, and 30% WSB (WSB10, WSB20, and WSB30, respectively) in exchange for corn gluten meal and rice in the base diet (WSB0). Six highly trained panelists evaluated the kibble's appearance, aroma, flavor, aftertaste, and texture of the experimental diets. Consumer acceptance data were collected from 94 qualified participants based on appearance and aroma. Color and fracturability increased ($P < 0.05$), whereas porosity, gritty, oily mouthcoating, and heated oil aftertaste decreased ($P < 0.05$) as WSB inclusion level increased. Consumers' acceptance scores did not change except for color. Based on these results, it is likely that consumers would not reject samples with WSB up to 30% based on overall liking, appearance, size, shape, aroma, or perceptions of their dog's liking. Thus, increasing WSB level in dog food up to 30% was not detrimental for most descriptive sensory properties of consumers' acceptance.

6.2 Introduction

Soybeans are composed of various biologically active compounds that provide important health benefits. These compounds can be classified into five parts: proteins, lipids, carbohydrates, minerals, and minor compounds. Soybeans are a source of protein that can serve as replacement of animal proteins. They contain storage proteins, trypsin inhibitors, and some lectins that lower cholesterol in blood and prevent cancer (Friedman et al., 2001; Sugano 2006). The lipids in soybeans include linoleic acid, α-linolenic acid, tocopherols, phytosterols, phospholipids, and sphingolipids, which have hypotriglyceridemic and cardiovascular effects, antioxidant properties, reduce fat accumulation in the liver and maintain brain functions (Olivera et al., 2005; Kritchevsky et al., 2005; Wang, 2008). Soybean carbohydrates mainly include the complex polysaccharides cellulose, hemicellulose, and pectin, the disaccharide sucrose, and the tetrasaccharide stachyose. Dietary fiber provides antihypertensive effects, improves digestive tract function, prevents colon cancer, and has hypotriglyceridemic and hypocholesterolemic effects (Sugano, 2006; Anderson et al., 2009). The minerals in soybeans include calcium, magnesium, phosphorus, and potassium, which support basic functions such as energy production, growth, healing, and proper utilization of vitamins and other nutrients (Galanakis, 2016). The minor constituents include phytic acid and isoflavones, which act as antioxidants, have estrogenic activity, and lower cholesterol in blood when fed in combination with soy proteins.

Although studies have demonstrated the benefits of soybeans, some pet food purchasers consider them to be a poor-quality ingredient due to underlying negative perceptions of soybeans spread through social media outlets (Your Dog Tufts, 2015; Henriques, 2022). Whole soybeans (WSB) contain 38.5% crude protein, 20.9% crude fat, and 19.8% total dietary fiber (dry matter basis; Kim and Aldrich, 2023) which should qualify

WSB to be a nutritionally excellent ingredient for dogs. The anti-nutritional factors in WSB such as trypsin inhibitors can be deactivated by heat under normal extrusion processes, which is the most common method for producing dry pet food (Bentum, 2021).

The palatability of soybeans in foods has been evaluated by dogs previously (Félix et al., 2012 and Kim et al., 2023). In these studies, dogs preferred soybean meal up to 300 g/kg in exchange for poultry offal meal (Félix et al., 2012). Dogs also preferred diets containing soybeans up to 300 g/kg as a replacement for corn gluten meal and chicken fat due to their aromatic attributes (Kim et al., 2023). Pet owners are often asked describe their pets' food palatability in terms of their animal's liking behaviors because pet owners "know" their animals' preferences well (Knight and Satchell, 2021). Although some pet owners may avoid soybeans due to the negative biases and unfounded perceptions from social media, they may miss out on the positive aspects regarding palatability for their dogs.

Descriptive sensory analysis by trained human panelists may help translate pet food preferences of pet parents into insights for research and development (Koppel, 2014). So far, there is a lack of information and research regarding consumer acceptance of soybean-containing dog foods which supports the negative perceptions of soybean-containing diets. Therefore, the objectives of this paper were to understand the effect of different inclusion levels of whole soybeans (WSB) in dry dog foods on descriptive sensory properties and consumer acceptance.

6.3 Materials and methods

6.3.1 Diet production and sample preparation

Four experimental diets were formulated to be nutritionally adequate for adult dogs (AAFCO, 2020a). Brewer's rice, corn gluten meal and chicken fat in the control, with no soybean (WSB0) were replaced by WSB at 10% (WSB10), 20% (WSB20), and 30%

(WSB30) of the formula (Table 6.1). Detailed diet production conditions were described by Kim and Aldrich (2023). Briefly, the dry expanded pet foods were produced using a single screw extruder (model E525, ExtruTech, Inc., Sabetha, KS, USA). The amount of surface applied chicken fat decreased with each increment of added soybeans to maintain a constant dietary fat level with a minimum topical fat > 2.0 % typical for commercial pet food production. The diets were stored frozen (-20 °C) and thawed at room temperature one day before the analysis.

6.3.2 Descriptive analysis

A total of four samples from each of the dry extruded dog food treatments (WSB0, WSB10, WSB20, WSB30) were evaluated using descriptive sensory analysis. The dog food samples (5 g) were served in 12 oz. Styrofoam cups for appearance and aroma evaluation; and 3.25 oz. plastic cups for flavor, aftertaste, and texture evaluation. Each container was labeled with a random three-digit code to prevent any bias from the panelists. The samples were delivered to the panelists due to the COVID-19 safety protocols and the analysis was conducted individually.

This project involved the participation of six highly trained panelists from the Center for Sensory Analysis and Consumer Behavior at Kansas State University. Each panelist had received more than 120 hours of general descriptive analysis training and over 1,500 hours of descriptive sensory experience, which included testing pet foods. Research has shown that a smaller panel consisting of highly trained individuals is better suited for discriminating among samples, compared to larger panels consisting of less trained individuals (Di Donfrancesco et al., 2012; Yang et al., 2021). The panelists rated the intensity of aroma including overall intensity, heated oil, fish, brothy, grain, cardboard, vitamin, and metallic; flavor attributes including grain, heated oil, vitamin, brothy, cardboard, salt, bitter, and metallic; aftertaste attributes including grain, bitter, and heated oil; texture attributes

including grittiness, fracturability, tooth packing, particle amount, oily mouthcoating, and hardness; and appearance characteristics such as color and porosity (Di Donfrancesco et al., 2012). To measure the intensity of each attribute, a numeric scale of 0-15 with 0.5 increments was applied, where 0 represents none and 15 represents extremely high intensity. Panelists had warm deionized water, mozzarella cheese, cucumber slices, unsalted crackers, and wash cloths to cleanse their palate before moving to the next sample analysis. The samples were evaluated in triplicate in a randomized order. The test utilized a home use test methodology, which means that the testing was conducted in the homes of each panelist.

Descriptive analysis data was collected using RedJade software (RedJade ®, Redwood Shores, CA, USA) and analyzed with 1-way analysis of variance (ANOVA) using a sensory analysis statistical tool (XLSTAT Sensory; Addinsoft, Paris, France) to determine the significant differences among treatments on each attribute. Differences were assessed using Fisher's protected Least Significant Difference and considered significant at $P < 0.05$.

6.3.3 Consumer study

The consumer study was conducted as a home use test due to COVID-19. Consumers were recruited through the database of the Sensory and Consumer Research Center (Manhattan, KS, USA) via email solicitation. A total of 94 consumers were qualified for a Home Use Test (HUT) by completing the online screening through RedJade software (RedJade ®, Redwood Shores, CA, USA). Qualified consumers were a) dog owners above 18 years old, b) without any health problems or food allergies, and c) frequent to feed or purchase dry dog foods for their dogs. The dry dog food samples were placed into an 8 oz Styrofoam bowls covered with lids and identified with a random three-digit code to prevent any bias from the consumer panels. The participants were given the samples and an instruction paper regarding the test procedure to be completed at home and timeline from the Sensory and Consumer Research Center (Manhattan, KS, USA). The consumers were

instructed to assess four distinct dog food samples, each to be evaluated sequentially. Consumers were expressly advised against tasting any of these samples or allowing their dogs to taste the samples. Each participant was compensated ($30) after the test completion. The demographics of the participants are in Table 6.2.

Consumer study data was collected using an online survey system (Qualtrics; Provo, Utah, USA). Each participant received a questionnaire link by email. For each sample, questions of overall liking, appearance liking, aroma liking, and texture liking were evaluated based on the 9-point hedonic scale, where 1 indicated dislike extremely and 9 indicated like extremely. Dog liking was evaluated by consumers opinions on whether their dogs would appreciate the items, and it is important to clarify that these products were not actually fed to the dogs during this process. Just-about-right (JAR) scales were used to determine if the consumers think the intensity of the attributes are right. A 5-point scale from 'far too light (FTL)' and 'too weak (TW)' to 'far too dark (FTD)' and 'too strong (TS)' with mid-point 'just about right (JAR)' was provided for color and aroma, respectively.

Consumer study data was analyzed with 1-way ANOVA mixed effect model using SAS (version 9.4, SAS Institute, Inc., Cary, NC, USA). Consumers were segmented by age and monthly spend on pet foods. Data was assessed using pair-wise comparisons based on least square (LS) means. Penalty analysis was applied to JAR questions data to assess color acceptance and determine whether the consumers penalized the samples due to too high to too low color intensity. The criteria were considered significant at $P < 0.05$. Principal component analysis (PCA) was conducted to correlate descriptive sensory attributes to consumers' overall liking scores using XLSTAT (version 2017, Addinsoft, Paris, France).

6.4 Results

6.4.1 Descriptive analysis

Increasing levels of whole soybean resulted in changes in appearance and texture attributes, with a general trend of decreasing intensity for color, porosity, grittiness, fracturability, and oily mouthcoating (Table 6.3). Tooth packing, particle amount, and hardness showed no significant differences among treatments. Most aroma, flavor, and aftertaste attributes did not exhibit significant differences among the diets (Table 6.4). However, WSB20 diet had a significantly lower mean score (3.03) in heated oil aftertaste attribute compared to WSB0 (3.47) and WSB10 (3.42) diets. The WSB30 diet (3.14) had a mean score between WSB20 and WSB10, with no significant difference when compared to other.

6.4.2 Consumer study

The majority of participants were female (85.1%), and the participants ages were evenly distributed (51.1% between 18-40 years and 48.9% 40+ years). Most participants reported that they fed their dogs twice daily (71.3%). Monthly expenditures on dog food were less than $50 for 47.9%.

The mean scores for acceptability of the experimental diets including overall liking, appearance, color, size, shape, aroma, and dog's liking all exceeded a score of 5 (neither like nor dislike) and ranged from 5.13 to 6.4 (Table 6.5). Except for color acceptance scores, the scores were not different among treatments. Consumer-based dog liking scores on the WSB-containing diets ranged from 6.30 to 6.47, which were higher than consumers' own overall liking scores (range from 5.43 to 5.91). The color acceptability for WSB30 was lower ($P < 0.05$) than WSB0 and WSB10, leading to less than 5 for the mean acceptability scores when

WSB was included at 30%. As the color became lighter with increasing WSB inclusion, the consumer acceptance scores decreased.

The penalty analysis for color liking and aroma liking of the diets provided the percentages of participants thought the sample to be just about right (JAR) (Table 6.6). For color liking, WSB10 had the highest percentage of consumers (48.9%) reported as JAR, whereas WSB30 had the lowest percentage (27.7%) reported as JAR. The majority of people (71%) thought the color of WSB30 was far too light (FTL). The frequencies of FTL attitudes from consumers increased as the WSB inclusion level increased in the diets. For aroma liking, WSB10 had the highest percentage of consumers (58.5%) reported as JAR, whereas WSB 20 had the lowest percentage (52.1%) reported as JAR. The major reason for "not JAR" for consumers was that the aroma was too weak rather than too strong.

Although the biplot obtained by PCA (Figure 1) presented an overall picture of the descriptive sensory attributes and consumer's overall likings perceived per diet treatment, it is important to note that there were only four samples with slight differences. The components F1 and F2 explained 84.01% of the variation in the dataset. The biplot portrays a divergence in samples after WSB inclusion exceeded 10%, with WSB0 (Control) and WSB10 being closely characterized by color and porous appearance. Meanwhile, WSB20 and WSB30 exhibited more distinct sensory attributes, such as grain and brothy aroma for WSB20, and overall intensity aroma and heated oil aroma for WSB30. The overall consumer liking was spread out randomly toward various descriptive sensory attributes. However, there was a smaller number of consumers who had high overall liking to vitamin and metallic aromatic attributes.

6.5 Discussion

6.5.1 Descriptive analysis

Descriptive sensory analysis is the most powerful method for capturing characteristics of food products based on their perceived sensory attributes and intensities (Suwonsichon, 2019). Di Donfrancesco et al. (2012) developed an initial sensory lexicon for human description of the appearance, texture, aroma, and flavor attributes that is specialized for assessing dry dog foods using human taste panels. Although dogs have far greater olfactory capability than humans (Kokocińska-Kusiak et al., 2021), the flavor profiles created by human panelists enable researchers to predict the effects of ingredients changes on the extruded dry pet foods (Pickering, 2009). In the current study, whole soybean inclusion level increased from 0 to 30% in exchange for corn gluten meal, rice, and chicken fat in the formulas. The sensory characteristics affected by the whole soybean inclusion levels included color and porosity for the appearance attributes, gritty, fracturability, and oily mouthcoating for the texture attributes, and heated oil for aftertaste attributes.

The decreased color intensity of products from increased whole soybeans may have been due to two key reasons: dilution of carotenoid pigments in the corn gluten meal (Cha et al., 2000) as whole soybean increased and these pigments could have been degraded by high temperature during the process, and a decrease in per unit volume since density decreased with more soybeans, leading to the lighter color (Zhang et al., 2023). There was a decrease in color greenness and lightness when corn starch-based extruded snacks were fortified with bean flours (Anton et al., 2009), which is similar to what was observed in the current study. The other factor that could have affected the color intensity is Maillard reaction between reducing sugars and compounds containing amino groups under the heat extrusion processing that leads to the formation of brown products with darker colors (Knerr et al., 2001).

Temperature and pH are critical parameters that influence the Maillard reaction in food (Martins et al., 2000). Pęksa et al. (2016) reported that color of extruded corn snacks was affected by extrusion parameters, mainly from the extrusion temperature. There was a linear decrease in extruder die temperature as the whole soybean inclusion levels increased during experimental diet production (Kim and Aldrich, 2023), and this ingredient-influenced extrusion parameter could have affected the color appearance in the final products. On the other hand, findings by Serrem et al. (2011) showed high intensity of brown color in sorghum and wheat biscuits when fortified with defatted soy flour were caused by the production of brown polymers from the Maillard reaction.

Unlike humans which have three types of cone photoreceptor cells that can distinguish red, green, and blue color, dogs have only two cone photoreceptor cells that are responsible for blue and yellow color (Neitz et al., 1989). More research is needed to understand the extent of the dog's color vision but so far, dogs are known to be less sensitive to color perception than humans (Byosiere et al., 2018), however, color intensity that drives human consumers' acceptance may impact preference (or provision of a certain meal) in dog foods.

Extrusion processing conditions and the chemical composition of the formulas affect product density and product expansion, which affect texture of the products. Especially with high amounts of fat in whole soybean which lubricated the raw materials within the extruder, lowered the friction generated within the barrel, and resulted in the less specific mechanical energy with less expanded products (Kim and Aldrich, 2023). In addition, whole soybeans contain fibers which can lower the degree of starch gelatinization and viscosity of the mass within the extruder, leading to less expanded products with reduced porous structure (Pęksa et al., 2016). More expanded products are more structurally porous (Koppel et al., 2014) and may explain the lower porosity of the products when whole soybean increased. Fracturability

is defined as “the force with which the sample ruptures” and it decreased as the mechanical energy input decreased due to higher macromolecular degradation. The grittiness and fracturability decreased with increased whole soybean levels, though hardness was not affected. Hardness is defined as “the force required to bite completely through the sample with molar teeth.” According to Koppel et al. (2015), the hardness and fiber inclusion in diets are negatively associated with dogs’ palatability, whereas fracturability and initial crispness might be positively associated with palatability in dogs. Oily mouthcoating texture attribute decreased as the whole soybean inclusion increased, which is connected to the fact that the amount of chicken fat that was sprayed outside of the extrudates decreased to maintain the total fat content of the formulas.

The heated oil aftertaste decreased as the whole soybean level increased. Due to the fat content in the whole soybean, the ration that was heat processed through extrusion had different fat contents (Kim and Aldrich, 2023), resulting in less heated chicken fat applied to the extrudates than the higher whole soybean formulas. The decrease in heated oil aftertaste was consistent with the decreased oily mouthcoating texture and the food manufacturing process. Even though there were no perceived differences in aroma or flavor attributes among treatments by human panelists, dogs could potentially recognize the differences because dogs have much more sensitive sense of smell (Kokocińska et al., 2022). From the work by Kim et al. (2023), dogs favored whole soybean containing diets for the first-choice measurement which is related to the aromatic characteristics of the food, suggesting dogs perceived aromatic differences among the diets.

6.5.2 Consumer study

The influence of dog owners on their pets' food preferences is substantial, as they are not only responsible for purchasing decisions but also closely observe their dogs' reactions while consuming the products. Moreover, dogs have been shown to be susceptible

to their owners' preferences when selecting food, even in tasks that involve discriminating between quantities. This highlights the significant impact that human perception of dog food has on the dogs' liking of the products (Prato-Previde et al., 2007). Ingredients have been identified in multiple studies to be the most important factor for most pet owners when selecting pet food (Schleicher et al., 2019). Whole soybeans are nutritive ingredients rich in protein and fat that are essential to dogs. The perceptions shaped by consumers from social media are not positive; wherein, soybeans are regarded as cheap and inferior replacement for meat and have anti-nutritional factors and compounds that lead to digestive upset and flatulence (oligosaccharides). However, there are many published studies that have evaluated soybeans as valuable ingredient in dog diets (Yamka et al., 2003; Purushotham et al., 2007; Félix et al., 2013; Kim et al., 2023). In addition, the pet food industry is beginning to embrace more ‘plant-based,’ ‘vegetarian,’ and ‘sustainable’ dog foods. There are about 20 million vegetarian pet owners in the US and 45% of these pet owners (including non-vegetarian pet owners) expressed a desire to feed a plant-based diet if one were available that met their criteria (Dodd et al., 2019). Whole soybean can be a suitable ingredient for this demand. In the current consumer study, the inclusion of whole soybeans in the formulas was not revealed to the participants to prevent bias from the consumer panels. The results suggest no bias in the current circumstances from a visual or aroma perspective.

Whole soybean inclusion levels (up to 30%) did not have a significant effect on consumers acceptance except for color. It was previously reported that the pet owner’s overall liking score of a dog food was influenced more by the appearance of the sample, especially the color, than the aroma of the product (Di Donfrancesco et al., 2014). A study by Gomez Baquero et al. (2018) found that the consumer acceptability of dry dog foods was rated highest when the samples were single kibbles of medium size, traditional shapes (such as triangular), and brown colors (golden brown or medium brown). The consumers rated

lowest for color liking when the single kibbles were red, green, or light brown. Further, the decrease in color liking scores from consumers can be related to the decrease of the color intensity of the products as detected by descriptive analysis. The dog's liking score perceived by the owners was maintained high (6.40 ± 1.59). This suggests that consumers believed all dog food samples with WSB included would be accepted by their dogs when compared with WSB0 samples. Unlike cat owners, who are more strongly influenced by the appearance and smell of pet foods, dog owners focused more on the meat presence in the diet and a healthy stool appearance (Vinassa et al., 2020). The reason why dog owners worry less about their dog's food palatability could be that dogs in general tend to be more food motivated and are generally known to devour the food when offered, whereas cats tend to be more finicky and may display more reluctant or selective consumption behavior.

The limitation of the current study was that we only studied the product effects without brands, prices, or health-related claims regarding the diets. We also did not collect the dogs' liking response when the consumers fed the diets to them but only collected the dogs' liking score based on the consumers' assumptions. Future research using an in-home test with questionnaires to score their dogs' response to the diets perceived by the dogs' owners may be helpful to get more practical consumer liking scores for dog food products. There have been several novel approaches using animal behaviors to evaluate the palatability of foods, but this behavior has not been deciphered into quantifiable methods of analysis yet (Tobie et al., 2015; Aldrich and Koppel, 2015). Furthermore, because dog owners cared about healthy stool appearance of their dogs (Vinassa et al., 2020), stool quality or digestibility of the diets can be a tool that reflects some of the consumers' preferences to the products. In addition, further analysis to evaluate volatile compounds from the diets to investigate associations between the chemical compounds to descriptive analysis or consumer liking might reveal more insights.

6.6 Conclusions

This study applied descriptive sensory analysis and consumer acceptance test to uncover the effects of whole soybean inclusion in dog foods on the sensory properties of the products. Overall, whole soybeans could be a good resource to replace proportional levels of brewers' rice, corn gluten meal, and chicken fat in dog foods and could be acceptable for dogs and consumers. Only a slight change in color liking was observed as the whole soybean levels increased; however, consumers still responded favorably to their dogs' liking scores for the diets. Further study using a home test to let the dog owners evaluate their dogs' liking scores while they offered the diets may be helpful to get more practical dogs' liking scores perceived by human consumers.

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6.8 Chapter 6 Figures

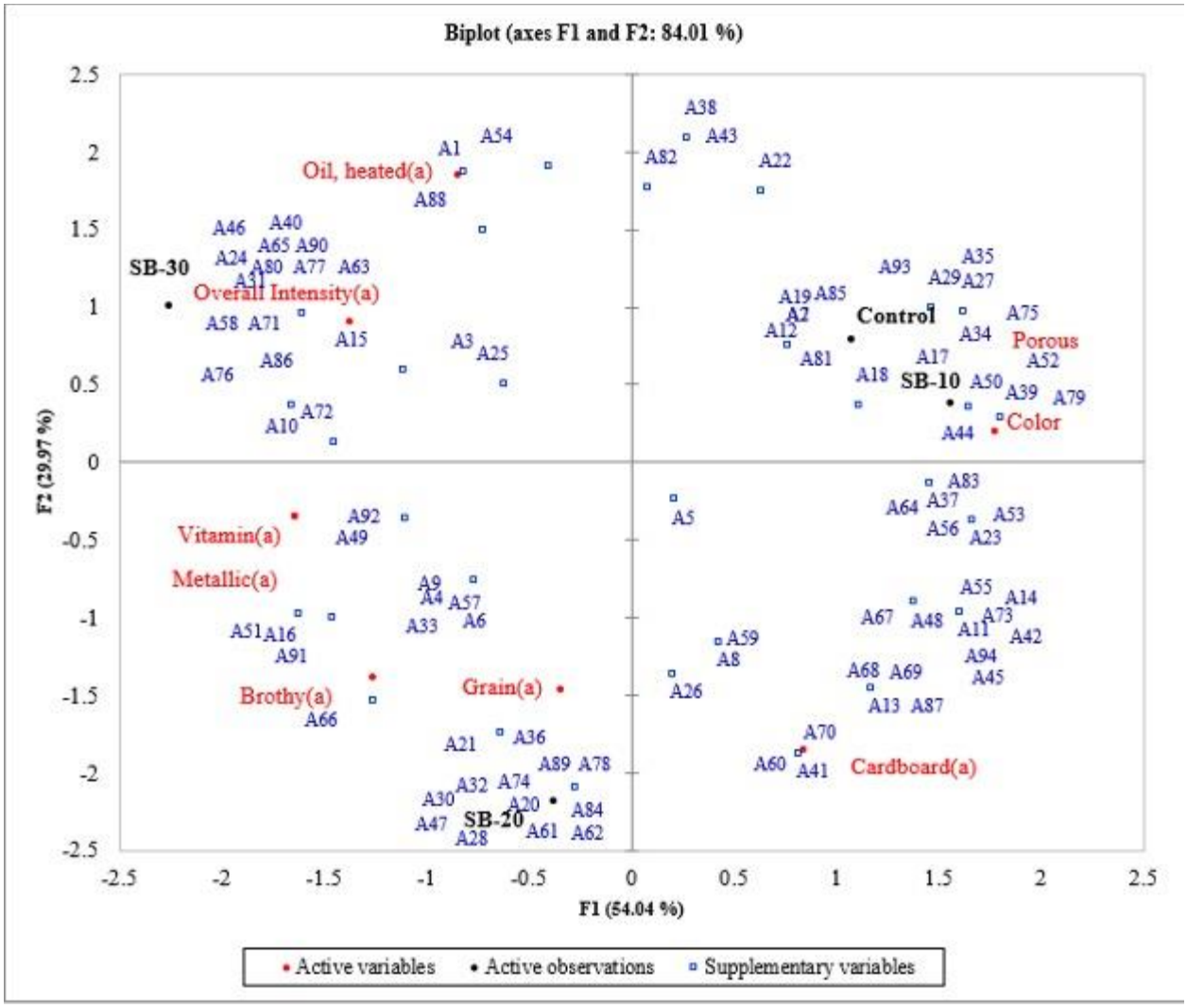

Figure 6.1 Principal component analysis biplot of descriptive sensory attributes (appearance and aroma) and consumers overall liking of 4 experimental diets with increasing levels of whole soybeans (Control, WSB0%; SB-10, 10%; SB-20, 20%; and SB-30, 30%)

6.9 Chapter 6 Tables

Table 6.1 Diet formulations with calculated nutrient compositions of the experimental diets with increasing levels of whole soybeans (WSB)

Ingredient, %	WSB0[4]	WSB10	WSB20	WSB30
WSB	0.00	10.00	20.00	30.00
Corn	22.50	22.50	22.50	22.50
Wheat	22.50	22.50	22.50	22.50
Corn gluten meal, 60%	15.74	9.54	3.55	0.00
Chicken meal	15.00	15.00	15.00	15.00
Rice, Brewers	8.58	6.67	4.54	0.00
Beet pulp	4.00	4.00	4.00	4.00
Salt	0.50	0.50	0.50	0.50
Dicalcium phosphate	0.45	0.45	0.45	0.45
Titanium dioxide	0.40	0.40	0.40	0.40
Potassium chloride	0.25	0.25	0.25	0.25
Choline chloride, 60% dry	0.20	0.20	0.20	0.20
Fish oil	0.20	0.20	0.20	0.20
Calcium carbonate	0.15	0.15	0.15	0.15
Vitamin premix[1]	0.15	0.15	0.15	0.15
Flaxseed	0.13	0.13	0.13	0.13
Trace Mineral Premix[2]	0.10	0.10	0.10	0.10
L-Threonine 98%	0.10	0.10	0.10	0.10
Dry natural antioxidant[3]	0.04	0.04	0.04	0.04
Chicken fat (topical)	8.02	6.13	4.25	2.34
Digest - dry dog flavor (topical)	1.00	1.00	1.00	1.00

[1]Vitamin premix: 5.51% moisture, 4.02% crude protein, 34.5% ash, 13.4% calcium, 17,162,999 IU/kg Vitamin A, 920,000 IU/kg Vitamin D, 79,887 IU/kg Vitamin E, 14,252 mg/kg thiamine, 4,719 mg/kg riboflavin, 12,186 mg/kg pantothenic acid, 64,736 mg/kg Niacin, 5,537 mg/kg pyridoxine, 720 mg/kg Folic acid, 70 mg/kg biotin, 22 mg/kg vitamin B12.

[2]Trace mineral premix: 0.66% moisture, 21.5% calcium, 0.02% sodium, 0.57% magnesium, 38,910 mg/kg iron, 11,234 mg/kg copper, 5,842 mg/kg manganese, 88,000

mg/kg zinc, 1,584 mg/kg iodine, 310 mg/kg selenium, 19% carbohydrate, and 1% crude fat.

[3]Dry natural antioxidant: mixed tocopherols, citric acid, rosemary extract, and soybean oil.

[4]WSB0, 0% whole soybeans; WSB10, 10%; WSB20, 20%; and WSB30, 30%

Table 6.2 The demographics of the participants and their dog feeding frequency in home use test, n=94

Dog owner characteristics	Frequency	Percentage, %
Gender		
Male	14	14.9
Female	80	85.1
Age		
18-40	48	51.1
40+	46	48.9
Feeding dog frequency		
Twice or more than twice every day	4	4.3
Twice every day	67	71.3
Once every day	15	16.0
Other	8	8.4
Expenditure on dog food, monthly		
Less than $50	45	47.9
$50 – 99	46	48.9
Above $99	3	3.2

Table 6.3 Mean scores of intensities[1] for descriptive analysis appearance (A) and texture (T) attributes of the experimental diets with increasing levels of whole soybean (WSB0, 0%; WSB10, 10%; WSB20, 20%; and WSB30, 30%)

	WSB0	WSB10	WSB20	WSB30
Color (A)	2.97[a]	3.03[a]	2.75[a]	2.31[b]
Porosity (A)	5.25[a]	4.33[b]	4.03[b]	3.72[b]
Grittiness (T)	7.75[a]	7.22[a]	6.53[b]	6.25[b]
Fracturability (T)	9.72[a]	9.89[a]	8.97[b]	7.33[c]
Tooth packing (T)	2.89	2.64	3.39	3.22
Particle amount (T)	4.11	4.08	4.22	4.53
Oily mouthcoating (T)	2.81[a]	1.94[b]	2.33[ab]	1.11[c]
Hardness (T)	9.75	10.33	10.33	10.42

[1]Intensity was measured using a numeric scale of 0-15 with 0.5 increments.

[abc]Within a row, means without a common superscript differ ($P < 0.05$).

Table 6.4 Mean scores of intensities[1] for descriptive analysis: aroma, flavor (f) and aftertaste (af) of the experimental diets with increasing levels of whole soybeans (WSB0, 0%; WSB10, 10%; WSB20, 20%; and WSB30, 30%)

	WSB0	WSB10	WSB20	WSB30
Overall Intensity	7.31	7.17	7.25	7.67
Oil, heated	4.22	4.11	4.08	4.28
Brothy	2.42	2.31	2.56	2.47
Grain	4.83	4.61	4.92	4.81
Cardboard	3.00	3.19	3.22	2.97
Vitamin	2.42	2.47	2.61	2.72
Metallic	1.89	1.94	2.00	2.08
Grain (f)	6.36	6.39	6.61	6.64
Oil, Heated (f)	4.78	4.69	4.39	4.86
Vitamin (f)	2.31	2.14	2.39	2.50
Brothy (f)	2.53	2.50	2.69	2.69
Cardboard (f)	3.00	2.97	2.81	2.83
Salt (f)	3.33	3.39	3.14	3.33
Bitter (f)	3.72	3.64	3.78	3.83
Metallic (f)	1.78	1.81	1.89	1.94
Grain (af)	5.31	5.31	5.47	5.50
Bitter (af)	3.67	3.47	3.72	3.69
Heated oil (af)	3.47[a]	3.42[a]	3.03[b]	3.14[ab]

[1]Intensity was measured using a numeric scale of 0-15 with 0.5 increments.

[ab]Within a row, means without a common superscript differ ($P < 0.05$).

Table 6.5 Mean scores[1] of acceptability of the experimental diets with increasing levels of whole soybeans (WSB0, 0%; WSB10, 10%; WSB20, 20%; and WSB30, 30%) (1: dislike extremely, 5: neither like nor dislike, 9: like extremely).

Sample	WSB0	WSB10	WSB20	WSB30	Mean ± Standard deviation
Overall liking	5.98	5.91	5.82	5.43	5.79 ± 1.61
Appearance	5.90	5.76	5.61	5.32	5.65 ± 1.79
Color	5.64[a]	5.36[a]	4.99[ab]	4.52[b]	5.13 ± 1.88
Size	6.31	6.02	6.39	5.95	6.17 ± 1.69
Shape	6.60	6.32	6.68	6.70	6.57 ± 1.48
Aroma	5.62	5.46	5.60	5.59	5.56 ± 1.66
Dog's liking	6.46	6.37	6.30	6.47	6.40 ± 1.59

[1]Scores were evaluated based on the 9-point hedonic scale, where 1 indicated disklike extremely and 9 indicated like extremely.

[ab]Within a row, means without a common superscript differ ($P < 0.05$).

Table 6.6 Penalty analysis on color liking and aroma liking of the experimental diets with increasing levels of whole soybeans (WSB0, 0%; WSB10, 10%; WSB20, 20%; and WSB30, 30%) (FTL; far too light, JAR; just about right, FTD; far too dark, TW; too weak, TS; too strong)

Sample	WSB0			WSB10			WSB20			WSB30		
Color liking												
Level	FTL	JAR	FTD	FTL	JAR	FTD	FTL	JAR	FTD	FTL	JAR	FTD
Frequencies	43	44	7	47	46	1	55	38	1	67	26	1
Percentage %	45.7	46.8	7.5	50.0	48.9	1.1	58.5	40.4	1.1	71.3	27.7	1.1
Mean	4.3	6.5	4.4	4.6	6.8	3	4.1	6.4	2	3.8	6.4	7
Mean drops	2.2		2.1	2.3		3.8	2.3		4.4	2.6		-0.6
Standardized difference	7.4			9.0			7.3			7.2		
Penalties		2.2			2.3			2.4			2.6	
Standardized difference		7.6			9.1			7.4			7.0	
Aroma liking												
Level	TW	JAR	TS	TW	JAR	TS	TW	JAR	TS	TW	JAR	TS
Frequencies	28	53	13	28	55	11	35	49	10	37	53	4
Percentage, %	29.8	56.4	13.8	29.8	58.5	11.7	37.2	52.1	10.6	39.4	56.4	4.3
Mean	5.1	5.9	4.3	5.1	6.2	3.9	5.1	6.3	3.8	5.1	6.2	2.5

Mean drops	0.8		1.6	1.1		2.3	1.2		2.5	1.1		3.7
Standardized difference	2.3			3.0			3.9			3.6		
Penalties		1.1			1.4			1.5			1.4	
Standardized difference		3.3			4.2			5.2			4.3	

Chapter 7 – Conclusions and recommendations

Soybean is the dominant oilseed in the U.S. Although soybeans have excellent potential to be a nutritious ingredient for dogs, their use in current pet foods is low. This research was conducted to explore alternative attributes of soy that might change the narrative for their inclusion in pet foods from a quality protein source to an economic fat and prebiotic source with processing benefits. The research found that whole soybeans were an excellent delivery vehicle for fat that increased the energy density of kibbles and provided gut health benefits for dogs. In conclusion, whole soybean inclusion at 10% in dog diets was the optimal level, and it was feasible to include both processing and dog acceptability at levels up to 30%. Questions remain regarding optimizing the extrusion processing conditions to eliminate the anti-nutritional factors in a whole soybean-containing diet and what the optimal level of soybeans is to capture the full advantage of their oligosaccharide content for pets. In addition, research that evaluates nutrient digestibility, hindgut fermentability, and palatability of soybeans in food intended for cats will be needed as well.