**Title: Determination of the dose and efficacy of market positive attributes from whole soy in extruded pet diets**

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**Executive Summary:**

A series of experiments was conducted to evaluate increasing levels (0, 10, 20, and 30%) of whole soybeans (WSB) in extruded dog diets. There was a linear increase in bulk density as increasing levels of WSB were incorporated into the ration under fixed processing parameters. The Trypsin inhibitor was diminished in all treatments from processing but was not completely eliminated. Digestibility by dogs declined in a slight linear fashion with increasing increments of WSB, but stool consistency and quality were not affected. In palatability testing dogs preferred the diets with WSB over that without, and in sensory analysis no large or technical differences or aversions were noted by the panelists. The in vitro fermentation of Soy oligosaccharides (OS) was comparably fermentable to beet pulp (a standard in the industry) and may provide a prebiotic effect to gastrointestinal health. In conclusion, adding WSB at incremental levels in pet food was able to increase internal fat levels in extruded foods, maintained digestibility and had better palatability than the no-WSB control, and provided fermentable OS which might benefit gastrointestinal health in dogs.

**Full Summary:**

As the petfood market continues to shift toward more premiumization and use of more whole ingredients with nutrition-health related messages, what is lacking for soy is information that demonstrates its unique “premium” value proposition to the pet food manufacturer and the pet owner for their companion animal. Specifically, the oligosaccharides and non-starch polysaccharides in whole soybean may actually be a positive for the pet by functioning as a prebiotic soluble fiber rather than a negative limiting factor. Also, with the inherent load of oil in whole soybeans, it might provide a new avenue to increase the energy density of the diet for dogs and cats without negatively affecting the processing or product integrity. Increasing the amount of liquid fat applied to the ration during extrusion can negatively affect product density and expansion. Therefore, adding whole soybeans into the formula may avoid production issues and decrease the amount of fat that has to be coated onto the surface of the product. The latter of which could benefit to shelf-life, owner acceptability (dryer, less greasy product), and improve the overall load of linoleic acid to the product.

In this study, whole soybeans (WSB) were characterized for their chemical composition regarding the dry matter, organic matter, crude protein, acid hydrolyzed ether extract, total starch, total dietary fiber (TDF), gross energy, trypsin inhibitor, urease, phosphorous and phytic acid, and oligosaccharides (sucrose, raffinose, stachyose, and verbascose) by standard methods. The nutrient composition of the WSB used for this study was consistent with expectations, with protein and fat exceeding 38% and 20%, respectively. The WSB was relatively high in TDF and low in starch. The anti-nutritional factors for the WSB were consistent with expectations; wherein, the trypsin inhibitor (TI) was high (>16,000 TIU/g), with moderate levels of urease activity, phosphorus, and phytic acid. The raw WSB contained significant amounts of oligosaccharides which corresponds to the previous literature. The WSB contained 35.72% of sucrose, 5.06% raffinose, 34.59% stachyose, and 0.01% verbascose on an as-is basis.

The objective of the extrusion processing study was to determine the effect of WSB on extrusion and product parameters for dry expanded dog food. Experimental diets were extruded with 0%, 10%, 20%, and 30% WSB (WSB0, WSB10, WSB20, and WSB30, respectively) in exchange for corn gluten meal and rice. Fixed processing inputs in the preconditioner were cylinder speed (185 rpm), water flow (58.7 ± 0.24 kg/h), and steam flow (53.3 ± 0.31 kg/h). Fixed processing inputs in extruder were extruder screw speed (425 rpm), extruder water and steam (0 %), die knife speed (700 rpm), and die size (9.5 mm diameter). Output parameters were measured at the preconditioner, extruder, and kibble. Measurements were collected at uniform time increments during the production of each experimental diet and considered treatment replicates. The trypsin inhibitor, urease, phosphorous, and phytic acid were analyzed with WSB, rations, and processed diets (WSB0, WSB10, WSB20, and WSB30, respectively). The least-square means of output parameters were analyzed with a single degree of freedom contrasts and significance at α = 0.05. As WSB increased, discharge temperature from the preconditioner increased linearly (*P* < 0.05). The extruder load, extruder die pressure, die exit temperature, and SME decreased linearly (*P* < 0.05) as WSB increased in the diets. Wet bulk density of the diets out of the extruder increased linearly (*P* < 0.05) while the wet kibble diameter and wet piece sectional expansion index (SEI) decreased linearly (*P* < 0.05) as WSB increased in the diets. Dry bulk density and moisture content of the diets out of the dryer increased linearly (*P* < 0.05) while the dry kibble diameter, width, and dry piece SEI decreased linearly (*P* < 0.05) as WSB increased in the diets. The trypsin inhibitor, urease, and phytic acid numerically increased in rations as the WSB inclusion level increased. After processing, trypsin inhibitor and urease decreased relative to before, but trypsin inhibitor was not completely eliminated, and phytic acid remained. In summary, the increased inclusion level of WSB changed processing conditions and outputs by the lubrication effect with their internal fat contents. In future work, it would be worth exploring whether increasing energy input in processing might decrease the bulk density and destroy anti-nutritional factors completely with the inclusion of WSB in diets.

The effects on the process were followed by animal evaluation. The objective of this study was to determine the effect of increasing levels of dietary WSB (noted above) on nutrient digestibility and stool quality by dogs. Eight castrated male and four spayed female Beagles of similar age (6.25 ± 0.452 years) were individually housed and fed the experimental diets. The study was designed as replicated 4 × 4 Latin square where dogs were allowed 9-d adaptation followed by 5-d total fecal collection for each period. Titanium dioxide was added to all diets (0.4%) to serve as an indigestible dietary marker to determine apparent total tract digestibility (ATTD). Least-square means of fecal parameters and ATTD were analyzed with a single degree of freedom contrasts and significance at α = 0.05. There was no difference among treatments for food intake or fecal scores. However, there were linear increases (*P* < 0.05) in DM fecal output, as is fecal output, fecal moisture, and defecation frequency (stools/day) as WSB increased in the diets. Apparent total tract digestibility of dry matter, crude protein, acid hydrolyzed ether extract, and gross energy decreased linearly as dogs were fed increasing levels of WSB in the diets (*P* < 0.05). While differences with increasing WSB inclusion were noted, the dogs remained healthy throughout the study, no adverse dietary reactions were noted, and the changes to utilization were not substantial. However, as noted in processing additional thermal energy either before or during extrusion may improve overall nutrient digestibility.

Palatability was determined with a 2-bowl test by beagle dogs (n = 20) for 2 days with each WSB diet compared to the WSB0 at an external commercial kennel. First choice preference and total food consumption were recorded for each dog, and individual intake ratios (IR) were calculated (intake of each diet/total intake). First choice (FC) was analyzed by a Chi2 probability, and the consumption of each diet was compared by a Wilcoxon signed rank test and a 2-way analysis of variance (2-way ANOVA). Dogs had greater (*P* < 0.05) first choice for WSB diets relative to the WSB0, though no difference among treatments for the food consumption and intake ratio were observed. In short, palatability was not affected by the increasing inclusion of WSB in dry dog food.

Regardless of what the dogs tell us, the pet owner is the one making the purchasing decision. Thus, a descriptive sensory analysis study was conducted to understand whether inclusion levels of WSB in dry dog foods had an impact on consumer (pet owner) sensory properties. Six highly trained panelists with experience evaluating pet foods participated in the descriptive analysis study. The panelists rated the intensity of aroma including overall intensity, heated oil, fish, brothy, grain, cardboard, vitamin, metallic; flavor attributes including grain oil, heated, fish, vitamin, brothy, cardboard, salt, bitter, metallic; texture attributes including gritty, fracturability, tooth packing, particle amount, oily, mouthcoating, hardness/firmness; and appearance characteristics such as brown color, porous, and flecks. For the evaluation, a numeric scale of 0-15 with 0.5 increments where 0 represents none and 15 represents extremely high was applied to each attribute to provide a measure of intensity. The samples were evaluated in triplicate in a randomized order. Descriptive analysis data was collected using RedJade software (RedJade ®, Redwood Shores, CA, USA) and analyzed by 1-way analysis of variance (ANOVA) (XLSTAT Sensory, Addinsoft, Paris, France) to determine the significant differences among samples on each attribute. Like the dogs, the panelists indicated that whole soybean inclusion levels did not have effects on the majority of sensory properties for these dog food samples. Those sensory elements affected included color and porous appearance attributes, gritty, fracturability, and oily mouthcoating for texture attributes, heated oil for aftertaste attributes.

As a follow-up, a GC-MS analysis sought to understand the effect WSB inclusion levels in dry dog foods might have on the volatile aromatic composition. The volatiles of the dry dog diet samples were evaluated using a gas chromatograph with a mass spectrometer (Shimadzu GC-2010 Plus, Kyoto, Japan). The GC-MS data was “cleaned” using R software (R Core Team). The code used was custom made for this application where each sample was read into the memory, the volatiles which were too low by area (< 20,000) and too high by timing (> 20 minutes) were removed and tabulated with all the other samples based on the same volatiles. Overall, 104 volatiles were detected out of which 37 were common in all treatments. The WSB0 had 11 unique volatiles and lacked 6 that were found in all other samples. Ethanol, 2-propoxy-; Heptan-2-yl formate; Octane, 2,6-dimethyl-; Tridecane; Nonane, 4-methyl-5-propyl- and Butane, 1,1,3,4-tetrachloro-1,2,2,3,4,4-hexafluoro- seem to be the volatiles that are introduced with adding the whole soybean. WSB10 had 12 unique volatiles and lacked 1 that was found in all other samples. WSB20 had 5 unique volatiles and lacked 2 that were found in all other samples. WSB30 contained 13 unique volatiles and lacked none that were found in all other samples.

Consumers were recruited from the Center for Sensory Analysis and Consumer Behavior (Manhattan, KS, USA) database and were contacted via email. A total of 94 consumers were qualified by completing the online screening through RedJade software (RedJade ®, Redwood Shores, CA, USA). The consumers were dog owners above 18 years old without any health problem or food allergy and frequent to feed dog(s) and purchase dry dog foods. In the test center, owners received a package with samples (in a paper bag marked with the owner’s participant code), and instructions. The questionnaire link was sent to each consumer by email. The owners were instructed on testing procedures and the timeline of the study. Each participant was compensated ($30) after completing the testing when they turned in the questionnaires. Consumer study data was collected using a Qualtrics online survey system. Each consumer received a questionnaire link. For each sample, questions of overall liking, appearance liking, aroma liking, and texture liking were included in the questionnaire. Consumer study data was analyzed by 1-way ANOVA mixed effect model using SAS (version 9.4, SAS Institute, Inc., Cary, NC) to determine significant differences among samples on each attribute and consumers. For all significant attributes, the sample effects were assessed using pair-wise comparisons based on least square (LS) means. The criteria for the significance at *P* < 0.05. Principal component analysis (PCA) was conducted in this study by XLSTAT (version 2017, Addinsoft, Paris, France). WSB inclusion levels did not have a significant effect on consumers’ acceptance of those dog food samples. When the consumers were segmented by ages (Above 40 and below 40), WSB inclusion did have a significant effect on their acceptance: people above 40 years had higher acceptance liking scores than the group of people below 40 years. Association analysis indicated that people who like WSB30 might like the overall intensity aroma and heated oil aroma. People who preferred WSB20 might like grain aroma and brothy aroma. People who preferred WSB0 and WSB10 might prefer color and porous appearance. But big differences were not observed and tended to confirm the limited differences noted by the dogs’ palatability and suggests there is little technical bias regarding the inclusion of soy in dog diets.

Finally, a fundamental understanding regarding features and benefits of WSB inclusion in diets may be related to the level and type of oligosaccharides in the diet. To help understand this question, an *in vitro* fermentation test was conducted to evaluate the OM disappearance, pH, and production of short-chain fatty acids. The comparisons differed from the dose-response study outlined above. In this case, fiber sources such as beet pulp (BP), pea fiber (PF), and soy hulls (SH) were used were compared to a TDF fraction of WSB alone or spiked with one of two oligosaccharide (OS) components. One source was an extract of soy oligosaccharides (WSBOS), and the other a blend of individually purchased OS (raffinose, stachyose, and verbascose; WSBRSV). Prior to the incubation, PF, BP, SH, and WSB samples were predigested using a Total Dietary Fiber Assay Kit (Neogen, catalog no. K-TDFA-200A) simulating the digestion in the small intestine of the dogs. The WSBOS treatment samples were prepared by mixing WSB TDF residues with WSB oligosaccharides (OS), which is a commercial product from Prairie AquaTech, based on the ratio of the actual TDF and total OS content of the raw WSB. The WSBRSV treatment samples were prepared by mixing each oligosaccharide (raffinose, stachyose, and verbascose) in a corresponding portion of the oligosaccharides analytical results from the raw WSB with WSB TDF residues (noted above). Fresh dog fecal samples were collected and maintained in anaerobic conditions until the dilution and inoculation. Test tubes containing the fibrous substrates were incubated for 0, 1, 2, 4, 8, and 12 h at 39 °C. At each incubation time point, subsamples were collected for fatty acids analysis. The remaining solutions were filtered on the following day and used to calculate OMD. Short-chain fatty acids (SCFA), branched-chain fatty acids (BCFA), and OMD were determined for each fiber source and time point. Compared to the BP, which is a gold standard as a moderately fermentable fiber, the WSBOS had higher OMD throughout all the time points from 0 to 12 h. The WSBRSV had lower OMD values than WSBOS throughout all the time points. The OMD of all the WSB treatments (WSBRSV, WSBOS, and WSB) increased linearly (*P* < 0.05) as the incubation time increased, throughout the time points from 0 h to 12 h. A quadratic response for pH of WSBRSV and WSBOS was also the lowest at 2 h to 4 h and started to increase as the incubation time increased. Thus, this would suggest that as hypothesized, the oligosaccharides could provide benefit to the incorporation of WSB by providing fermentable substrate as a standard fiber source such as BP. Future work should confirm these results with short chain fatty acid analysis, followed by a dose study to determine at what point the added OS are optimal for gastrointestinal health.

In conclusion, including whole soybeans at levels of 10 to 30% in pet food had a number of advantages that might provide new options for pet food companies to increase the use of this versatile and popular ingredient (human foods market) in modern pet foods.