

# Effects of cooked navy bean powder on apparent total tract nutrient digestibility and safety in healthy adult dogs<sup>1</sup>

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**ABSTRACT:** Dry beans (*Phaseolus vulgaris* L., Fabaceae) are a low glycemic index food containing protein, fiber, minerals, essential vitamins, and bioactive compounds and have not been evaluated for inclusion in commercial canine diets. The objective of this study was to establish the apparent total tract digestibility and safety of cooked navy bean powder when incorporated into a canine diet formulation at 25% (wt/wt) compared with a macro- and micro-nutrient matched control. Twenty-one healthy, free-living, male and female adult dogs of different breeds were used in a randomized, blinded, placebo controlled, 28-d dietary intervention study. Apparent total tract energy and nutrient digestibility of the navy bean powder diet were compared with the control diet. Digestibilities and ME content were 68.58 and 68.89% DM, 78.22 and 79.49% CP, 77.57 and

74.91% OM, 94.49 and 93.85% acid hydrolyzed fat, and 3,313 and 3,195 kcal ME/kg for the navy bean diet and control diet, respectively. No differences were observed between the groups. No increased flatulence or major change in fecal consistency was observed. Navy bean powder at 25% (wt/wt) of total diet was determined to be palatable (on the basis of intake and observation) and digestible in a variety of dog breeds. No changes were detected in clinical laboratory values, including complete blood counts, blood biochemical profiles, and urinalysis in either the bean or control diet groups. These results indicate that cooked navy bean powder can be safely included as a major food ingredient in canine diet formulations and provide a novel quality protein source, and its use warrants further investigation as a functional food for chronic disease control and prevention.

**Key words:** apparent total tract energy and nutrient digestibility, canine, dry beans

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J. Anim. Sci. 2012.90:2631–2638  
doi:10.2527/jas2011-4324

## INTRODUCTION

Staple foods are important determinants of health in humans and companion animals (Public Health Service, 1988; Hand, 2000). Developing nutritional guidelines to improve health and prevent disease in dogs has contributed to the production of numerous commercial

dog foods with novel carbohydrate (Fortes et al., 2010), fiber (Bednar et al., 2001; Swanson et al., 2001), and protein sources (Zentek and Mischke, 1997; Dust et al., 2005). Despite the success of plant-based nutrients in dog food, dry bean (*Phaseolus vulgaris* L., Fabaceae) is one staple food crop of global agricultural and nutritional importance that has been overlooked in commercial pet food formulations. Evidence supports that in addition to providing excellent sources of protein, fiber, minerals, essential vitamins, and bioactive compounds in greater concentrations than cereal grains, such as wheat and corn (Broughton et al., 2003), beans have chronic disease fighting properties, which slow or prevent disease progression (Geil and Anderson, 1994).

Navy beans were selected for this study because of their reported health benefits (Mentor-Marcel et

<sup>1</sup>The Shipley Foundation (Boston, MA), the Morris Animal Foundation (Denver, CO), Mark L. Morris Professorship at Texas A&M University, and the Animal Cancer Center Clinical Trials Core (Fort Collins, CO) provided funding for this work. The authors thank K. Arnett, A. Kumar, C. Ollila, and B. Barnett for providing technical assistance.

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Received June 1, 2011.

Accepted February 9, 2012.

al., 2009; Thompson et al., 2009) and availability in a cooked powder form. Navy bean consumption has not previously been examined in colony or companion canines. Digestibilities of the starch and fiber fractions of uncooked legumes were evaluated for canines in vitro and showed lower digestibility compared with other carbohydrate sources (Bednar et al., 2001). However, cooked beans have been successfully incorporated into homemade canine diets (R. L. Remillard, Veterinary Nutritional Consultations, Inc., Hollister, NC, personal communication) and soybeans, another legume, are digestible by dogs (Yamka et al., 2006).

The major objective of this study was to examine safety and digestibility of cooked navy bean powder in healthy dogs compared with a placebo control, commercial canine diet formulation. We hypothesized that cooked navy bean powder, as a major ingredient in an adult canine diet formulation, is palatable, safe, and digestible.

## MATERIALS AND METHODS

The Colorado State University Institutional Animal Care and Use Committee approved all clinical trial operations, animal care procedures, and collection of biological samples for safety and digestibility of experimental research diets before beginning the study.

### Study Design

Twenty-one healthy, adult, free-living dogs of different breeds were recruited to participate in a randomized, double-blinded, and placebo controlled canine dietary intervention study at the Animal Cancer Center of the Veterinary Teaching Hospital at Colorado State University. Dogs were randomized in a 1:1 manner for equal allocation to study groups and the study clinician determined a BCS during the baseline physical exam. Each dog received a study code number and both the owner and clinician were blinded to the assigned study treatment. Dogs were transitioned to the study diets over a 4-d period. Blood, urine, and feces were collected at the beginning and d 14 and 28 of the study. A 96-h pooled fecal sample was collected on d 15 to 19 of the study for digestibility analysis. On d 28, dogs were transitioned back to the original diet over a 4-d period.

### Inclusion or Exclusion Criteria for Canine Participation

Male and female dogs between the ages of 2 and 7 yr with BCS between 4 and 7 on a 9-point scale (Laflamme, 1997) and weighing at least 10 kg were qualified to participate in the study. Of the 21 dogs recruited, there were 10 different known breeds and several mixed breeds, and

**Table 1.** Breeds of 21 canine dietary intervention study participants in each treatment

Breed <sup>1</sup>	No. of dogs	
	Navy bean	Control
Australian Cattle Dog	2	2
Dalmatian	1	-
Hound mix	-	1
Mixed (unknown)	2	2
Pitbull mix	-	2
Pointer	1	-
Retriever (Golden/Lab)	1	3
St. Bernard	-	1
Standard Poodle	1	-
Terrier/Terrier mix	2	-
Total	10	11

<sup>1</sup>Breeds as reported by owners.

they were randomly distributed across treatment groups (Table 1). Dogs were excluded if they had any reported dietary allergies, intestinal sensitivities or discomforts, or prior history of cancer. Dogs must not have taken antibiotics or analgesics for at least 1 mo before starting the study. Heartworm prevention was allowed. Dog owners provided informed consent before the enrollment of their pets at the Colorado State University Animal Cancer Center. Participants were required to be present at the beginning and d 14 and 28 of the study, provide a 96-h fecal sample collection after consuming the study diets for 10 d, and record daily food intake and daily fecal scores for 28 d. Compliance with study protocol was determined by clinical trial coordinator with weekly phone calls to dog owners and during each study visit. Palatability and tolerance of the diet were determined using a pet health history survey that was completed by the owners on d 14 and d 28. Owners were asked about any incidence of vomiting, nausea, diarrhea, flatulence, changes in physical activity, appetite, and water intake, as well as any apparent changes in behavior.

### Canine Diet Formulations

Two canine diet formulations were used in this study that meet nutritional recommendations according to published feeding guidelines (AAFCO, 2010). A formula similar to an AAFCO (2010) approved, commercially available adult canine diet formula containing 27% CP and 12% crude fat was used for the 0% bean, placebo control. The control diet was mixed and manufactured under the same conditions and locations as the experimental bean diet (ADM Alliance Nutrition Feed Research Pilot Plant, Quincy, IL; Applied Food Biotechnology Plant in St. Charles, MO). The bean diet was formulated to match the control diet in macronutrient and caloric content, except for the inclusion of 25% cooked navy bean powder (Vegefull; ADM Edible Bean Special-

ties, Decatur, IL). Adjustment of major food ingredients, such as wheat and corn, were made to account for differences in the contribution of cooked navy bean powder to macro and micronutrients and total caloric contents. The fatty acid content of both diets was matched as well. Marine type long chain n-3 fatty acids were not present in either diet. The percentages of ingredients and chemical components are presented for each diet in Table 2. Table 3 presents the contribution of navy bean powder to the chemical composition of the experimental diet.

### Canine Diet Intervention

Dog owners were instructed to feed only the research diet provided by study clinical coordinator for the entire study duration and to measure out a prescribed amount of food for canine consumption each day. The prescribed daily energy consumption was determined by BW and according to the normal feeding habits of the dog (1 or 2 feedings daily). The total required daily energy intake for maintenance of each dog was calculated at the beginning of the study by this formula: daily ME requirement (kcal) =  $110 \times \text{BW}^{0.75}$ , where BW is in kilograms (NRC, 2006). The estimated energy intake was intended to maintain a stable BW in dogs for the study duration. An inappropriate BW change was defined by a change in BW of 2% per week or 4% change from each visit (AAFCO, 2010). Owners measured and recorded the volume of food offered and refused. The total amount consumed was calculated by subtracting the weight of the refused food from offered food. Water was provided ad libitum. The owner completed a daily intake record for 28 d, and a space was provided to record any intake aside from research diet that may affect study results.

### Blood and Urine Sample Collection

To assess the safety of feeding navy bean powder, overall metabolic status, and liver and kidney functions, blood, and urine samples were collected for blood diagnostic tests and urinalysis. Non-fasted blood samples were collected via jugular puncture at the beginning and d 14 and 28 of the study. At each visit, approximately 18 mL of blood was collected, and 1 mL of whole blood was collected into an evacuated red top tube without anticoagulant for biochemistry panel analysis. Another 1 mL of blood was collected into an evacuated lavender top tube containing EDTA for complete blood counts (CBC), hemoglobin (HGB), and hematocrit determination. The additional blood was used to isolate peripheral blood mononuclear cells and serum for future analyses. Urine samples were usually collected by the owner at home using provided specimen containers. In some in-

**Table 2.** Ingredient and chemical composition of cooked navy bean powder and control diets fed to 21 healthy, adult dogs<sup>1</sup>

Item	Navy bean, %	Control, %
Ingredient, % (as-fed)		
Navy bean (cooked, dehydrated)	25.00	-
Meat and bone meal	13.86	14.83
Brewer's rice	12.50	12.50
Corn	11.25	11.25
Corn gluten meal	9.35	14.24
Wheat middlings	8.27	14.50
Poultry fat	7.75	7.77
Poultry by product meal	6.50	6.50
Wheat grain	1.67	14.50
Beet pulp	1.00	1.00
Ground flaxseed	0.75	0.75
Salt	0.50	0.50
Brewer's yeast	0.50	0.50
Vitamin-trace mineral premix <sup>2</sup>	0.50	0.50
Monocalcium phosphate	0.39	0.08
KCl	0.10	0.30
L-Lys×HCl	-	0.22
Met	0.07	-
Choline chloride	0.05	0.05
Analyzed composition (as-fed)		
DM, %	94.28	95.01
n-6 Fatty acids, %	2.02	2.26
n-6/n-3 fatty acid ratio	10.06	10.91
LA/ALA ratio	9.79	10.54
(LA + ARA)/(ALA + EPA + DHA) ratio	9.93	10.7
AAFCO ME, kcal/kg	3,416	3,380
Analyzed composition (% DM)		
OM	91.83	91.37
Ash	8.17	8.63
CP	29.91	31.15
Acid hydrolyzed fat	13.58	14.00
Crude fiber	3.18	2.95
GE, kcal/kg	4,957	4,967

<sup>1</sup>LA = linoleic acid, ALA = alpha linoleic acid, ARA = arachidonic acid, EPA = eicosapentaenoic acid, DHA = docosahexaenoic acid, and AAFCO ME = ME based on AAFCO (2010).

<sup>2</sup>ADM Alliance Nutrition (Quincy, IL). Provided per kilogram of navy bean diet: vitamin A, 7,500 IU; vitamin D, 750 IU; vitamin E, 93.75 IU; thiamine, 3.75 mg; riboflavin, 30 mg; pantothenic acid, 12 mg; niacin, 15 mg; pyridoxine, 1.875 mg; folic acid, 0.26 mg; vitamin B12, 37.5 µg; choline, 1,700 mg; Fe from ferrous sulfate, 282 mg; Cu from copper sulfate, 15 mg; Mn from manganous oxide, 31 mg; Zn from zinc oxide, 187 mg; I from calcium iodate, 2 mg; and Se from sodium selenite, 0.7 mg. Provided per kilogram of control diet: vitamin A, 7,500 IU; vitamin D, 750 IU; vitamin E, 93.75 IU; thiamine, 3.75 mg; riboflavin, 30 mg; pantothenic acid, 12 mg; niacin, 15 mg; pyridoxine, 1.875 mg; folic acid, 0.26 mg; vitamin B12, 37.5 µg; choline, 1,711 mg; Fe from ferrous sulfate, 303 mg; Cu from copper sulfate, 16 mg; Mn from manganous oxide, 39 mg; Zn from zinc oxide, 196 mg; I from calcium iodate, 2 mg; and Se from sodium selenite, 0.7 mg.

stances, when the owner was unable to obtain a urine sample, ultrasound guided cystocentesis was used.

**Table 3.** Chemical composition of cooked, dehydrated navy beans (DM basis)

Component	Content
Nutrient, %	
Total carbohydrates	64.00
Sugars	4.02
CP	24.00
Acid hydrolyzed fat	3.00
Ca	0.26
K	1.00
P	0.40
Mg	0.13
Na	0.03
Thr	4.67
Cys	0.93
Val	5.60
Lys	6.73
Ile	4.77
Leu	8.37
Tyr	3.53
Phe	6.20
His	2.90
Met	1.20
Arg	6.27
Trp	1.10
AAFCO ME <sup>1</sup> , kcal/kg	3,344

<sup>1</sup>AAFCO ME = ME based on AAFCO (2010).

### Blood and Urine Analysis

The Clinical Pathology Laboratory at Colorado State University performed all blood and urine analyses. The biochemistry panel was analyzed using a clinical chemistry analyzer (Hitachi 917; Roche Diagnostics, Indianapolis, IN), and CBC was detected using an analyzer (Advia 120; Bayer, Tarrytown, NY). Urinalysis was measured using standardized clinical laboratory procedures. Color and clarity were assessed visually, specific gravity was determined using a refractometer with water as a reference. A chemstrip (Cobas Chemstrip 10 MD; Roche Diagnostics) was used to determine pH, protein, glucose, ketones, bilirubin, and blood concentrations. Any samples positive for protein were further analyzed with the sulfosalicylic acid turbidometric test (Exton's Method). Microscopic analysis of the urine sediment was used to determine and quantify cellular components, crystals, casts, and bacteria. All methods used have been previously described (Osborne, 1981).

### Fecal Scores and Sample Collection

Owners reported daily fecal scores using a 3-point scale with 1 = well formed, 2 = soft, and 3 = runny. A comment space was provided on the score sheet to obtain any observational changes per discretion of the owner. A 4-d (96-h) total fecal collection was performed for the measurement of apparent total tract macronutri-

ent digestibility after 10 d of consuming 100% of the experimental (placebo control or bean containing) diets. Samples were collected daily and stored at  $-20^{\circ}\text{C}$ . At the end of the collection period, the samples were weighed, pooled, and stored at  $-20^{\circ}\text{C}$  and then freeze-dried before proximate analysis.

### Proximate Analyses for Assessing Apparent Total Tract Nutrient Digestibility

Proximate analysis of both the research diets and the 96-h pooled fecal samples were performed accordingly: methods 935.29 for DM, 920.39 for fat, 942.05 for ash, and 990.03 for CP (AOAC, 2000). Organic matter was calculated by subtracting ash from DM or 100%. Gross energy was measured using an oxygen bomb calorimeter (Model 1261; Parr Instruments, Moline, IL). Crude fiber content was determined (Crude Fiber Method; ANKOM Technology, Macedon, NY), and samples were coded and blinded for proximate analyses (ADM Alliance Nutrition Laboratory, Quincy, IL). The lipid profile of the navy bean and control diets was determined according to previously reported protocols (Dunbar et al., 2010). Digestibility of protein, fat, OM, and DM were calculated by this formula, where nutrients were measured in grams on a DM basis: Nutrient digestibility (%) = [(nutrient intake – nutrient in feces) / nutrient intake]  $\times$  100. Metabolizable energy was calculated by this formula: ME (kcal/kg of food) = {GE of food consumed – GE of feces collected – [(g of protein consumed – g protein in feces)  $\times$  correction factor for energy lost in urine]} / grams of food consumed  $\times$  1,000 (AAFCO, 2010). Feed and fecal values on a DM basis were used in all calculations, and the correction factor for energy lost in urine was 1.25 kcal/g (AAFCO, 2010).

### Statistical Analysis

Data are presented as means and SEM. Non-paired *t*-test probabilities were used to determine differences in digestibility, nutrient intake, ME, fecal output, age, and BW means between the 2 diet groups. Blood results were analyzed in both diet groups and across time points using repeated measures of ANOVA. Within each time point, outliers were detected by a Grubbs test. Fisher's Exact Test was used for assessing differences in BCS and sex between diet groups. A probability of  $P < 0.05$  was accepted as statistically significant. Statistical analyses were performed using a software package (GraphPad Software, San Diego, CA).

## RESULTS

### Canine Participant Demographics

A diverse set of breed participants were recruited for this study to provide broad representation of the canine population. Table 4 shows the mean and SEM of age in years, BW in kilograms, median BCS, and sex by treatment of 21 dogs that participated in the study. No differences were observed between treatments at the beginning of the study.

### Peripheral Blood Measures

Serum alkaline phosphatase (ALP) concentrations in 1 control group dog were chronically greater than the normal range. Because there were no changes over the baseline value and no other abnormalities or clinical

**Table 4.** Comparison of age, BW, BCS, and sex of 21 canine dietary intervention study participants by diet

Item	Navy bean <sup>1</sup>		Control <sup>2</sup>		P-value <sup>3</sup>
	Mean	SEM	Mean	SEM	
Age, yr	4.1	0.5	3.2	0.4	0.17
BW, kg	23.4	1.5	28.2	3.3	0.21
BCS <sup>4</sup>					1.00
4 and 5, No. of dogs	8		9		
6 and 7, No. of dogs	2		2		
Sex <sup>5</sup>					0.67
Female, No. of dogs	6		5		
Male, No. of dogs	4		6		

<sup>1</sup>n = 10

<sup>2</sup>n = 11

<sup>3</sup>For age and BW, P-values were determined using a non-paired t-test. For count data, P-values were determined using Fisher's Exact test.

<sup>4</sup>Purina 9-point scale, where BCS 4 and 5 are ideal and 6 and 7 are overweight (Laflamme, 1997).

<sup>5</sup>All female participants were spayed and all but 1 male in the control group was castrated.

signs, the dog was allowed to remain in the study. Inclusion of data from this subject, however, increased the mean ALP value of the control group. The ALP values from this dog were determined to be outliers and were, therefore, excluded from the data analysis.

The results of blood diagnostic tests at the beginning and d 14 and 28 of the study indicated that there were no differences in HGB, packed cell volume (PCV), serum albumin concentrations, and ALP activities between treatment groups (Table 5). Average values for the navy bean and control diet groups on d 28, respectively, were 17.3 and 18.3 g/dL for HGB, 49 and 53% for PCV, 3.8 and 4.0 mg/dL for serum albumin, and 37 and 44 IU/L for ALP. In addition to these blood measurements required for assessing the nutritional adequacy of diets, complete blood cell count and biochemistry profiles, which included glucose, blood-urea nitrogen, creatinine, P, Ca, Mg, Na, K, Cl, total protein, globulin, albumin to globulin ratio, cholesterol, total bilirubin, creatinine kinase, alanine aminotransferase, aspartate transaminase, gamma-glutamyl transpeptidase, lipemia, and hemolysis were determined to assess safety (data not shown). No adverse changes were detected in any of the laboratory values examined between the treatment groups. The characteristics of the CBC and biochemistry panel were determined to be within normal ranges. Furthermore, no laboratory values were changed over the baseline values in any of the dogs.

### Urinalysis Results

As an additional safety measure, urinalysis was conducted at the beginning, d 14, and d 28 of the study, and all laboratory values, which included specific gravity, protein, bilirubin, ketones, blood, and crystal formation, were within normal ranges and no differences were observed between groups (data not shown). An intriguing trend was observed for urine pH such that the bean diet group had

**Table 5.** Blood characteristics at the beginning and d 14 and 28 of the study of 21 healthy adult canines fed the diet containing 25% cooked navy bean powder or control diet

Item <sup>1</sup>	Reference range <sup>2</sup>	Navy bean diet <sup>3</sup>						Control diet <sup>4</sup>						P-value
		Baseline		d 14		d 28		Baseline		d 14		d 28		
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
HGB, g/dL	13 to 20	17.4	0.4	17.3	0.7	17.3	0.3	17.8	0.3	18.0	0.4	18.3	0.4	0.15
PCV, %	40 to 55	51	1	50	1	49	1	51	1	52	1	53	1	0.13
Alb, mg/dL	2.5 to 4.0	3.8	0.1	3.8	0.1	3.8	0.1	3.9	0.1	4.0	0.1	4.0	0.1	0.16
ALP, IU/L	20 to 142	43	6	37	5	37	5	55	12	46	9	44	9	0.39

<sup>1</sup>HGB = hemoglobin, PCV = packed cell volume, Alb = serum albumin, and ALP = serum alkaline phosphatase. One dog in the control group was excluded due to chronically increased concentrations outside of the normal range.

<sup>2</sup>Reference ranges used at the Diagnostic Medicine Center, Colorado State University (Fort Collins, CO).

<sup>3</sup>n = 10.

<sup>4</sup>n = 11.

an average pH of 6.5 at the beginning, decreased to 5.5 at d 14, and then normalized to an average baseline value of 6.5 at d 28. The control group demonstrated an opposite trend where the average urine pH was 6 at the beginning, increased to 7 at d 14, and then returned to 6 at d 28. This transient response in urine pH between the bean diet and control diet groups may not have any statistical or clinical significance because both groups normalized at d 28, and there were no clinical changes in urate lithogenesis. The relevance of the acidic, yet transient, urinary response to bean consumption may be important for future clinical dietary bean investigations.

### **Nutrient Intake, BW, Digestibility, ME, and Fecal Characteristics**

The CP, fat, and OM intakes are reported on a DM basis, and no differences in macronutrient intakes, apparent total tract digestibility, fecal characteristics, or ME were observed between the bean and control diet groups (Table 6). Average intakes were 325.7 and 336.3 g DM, 97.4 and 104.7 g CP, 44.2 and 45.8 g fat, and 299.1 and 307.2 g OM/d for the navy bean and control diets, respectively. Average bean powder intake was 3.7 g/(kg BW·d). Total tract apparent digestibility and ME content were 68.58 and 68.89% DM, 78.22 and 79.49% CP, 77.57 and 74.91% OM, 94.49 and 93.85% acid hydrolyzed fat, and 3,313 and 3,195 kcal ME/kg for the navy bean diet and control diet groups, respectively. The total amount of fecal matter and fecal quality scores did

not change between the 2 groups. Fecal output average was 96.1 and 103.7 g/d for the bean diet and control diets, respectively. Owners most frequently reported well-formed stools. Two dogs, 1 from each group, increased BW by 4% on d 14 and the prescribed amount of food was reduced by 37.5 g for the bean diet and 32.6 g for the control diet per day. All other dogs maintained BW throughout the study duration. Owners reported a minimal incidence of food intake outside of the provided diet; however, these occurrences were equally distributed between the 2 groups. Isolated incidences of vomiting and diarrhea were reported in both groups, which were reported as unrelated to the diet by owner. None of the owners reported increased incidence of flatulence, and both diets were reported as equally palatable to all study participants on the basis of no differences in reported dietary intake or eating preferences as determined and reported by dog owners.

## **DISCUSSION**

The results reported herein demonstrated that cooked navy bean powder incorporated at 25% in an extruded dog diet is safe, palatable, and a digestible source of carbohydrates, protein, and fat for healthy, adult canines. Digestibility of selected commercial dry canine diets has been reported between 73.2 and 84.5% for DM, 77.2 and 87.8% for CP, 88.1 and 97.1% for fat, and 72.5% for OM in both dogs and other model systems (Brown, 1997; Krogdahl et al., 2004). Apparent total tract digestibility of the navy bean diet was similar to these values. Compelling findings of no changes in any of the blood and urine characteristics or systemic markers further substantiates safety or canine wellness after consumption of the navy bean diet. Of particular note in the urinalysis results was the lack of clinically relevant changes in crystal formation in any of the dogs consuming the navy bean diet, in spite of the transient change in urine pH, as legume consumption is contraindicated for dogs with increased risk for urolithiasis (Hand, 2000). These results in healthy canines provide a strong basis for future nutritional investigations of cooked bean powder intake in companion animals with chronic diseases, as beans are gaining widespread popularity for dietary disease prevention strategies in humans (Geil and Anderson, 1994; Messina, 1999; Kahlon and Woodruff, 2002; Papanikolaou and Fulgoni, 2008; Mitchell et al., 2009).

In addition to the novelty of including beans as a staple food ingredient for dogs, it is possible that beans may serve as a quality source of protein and fiber, provide a low glycemic index food, and deliver unique bioactive compounds for enhanced canine nutrition. Navy beans contain essential AA and may have greater bioavailability when compared with other plant protein sources.

**Table 6.** Daily intakes, apparent total tract digestibility, ME, and fecal characteristics of 21 dogs fed the diet with 25% cooked navy bean powder or control diet

Item	Navy bean <sup>1</sup>		Control <sup>2</sup>		P-value
	Mean	SEM	Mean	SEM	
Daily intake (DM basis)					
DM, g	325.7	19.1	336.3	30.6	0.78
CP, g	97.4	5.7	104.7	9.5	0.53
Acid hydrolyzed fat, g	44.2	2.6	45.83	4.2	0.76
OM, g	299.1	17.5	307.2	28.0	0.81
Apparent total tract digestibility, %					
DM	68.58	5.60	68.89	5.08	0.96
CP	78.22	3.90	79.49	3.52	0.81
Acid hydrolyzed fat	94.49	1.05	93.85	1.17	0.69
OM	77.57	3.81	74.91	3.30	0.60
ME, kcal/kg	3,313	164	3,195	150	0.60
Fecal characteristics					
Fecal output, g/d	96.1	14.4	103.7	15.8	0.72
Fecal score <sup>3</sup>	1	-	1	-	

<sup>1</sup>n = 10

<sup>2</sup>n = 11

<sup>3</sup>Fecal samples were scored according to this system: 1 = well formed, 2 = soft, and 3 = runny.

They may also provide less fat when compared with animal protein sources (Messina, 1999). Bean fiber was shown to be readily metabolized by human intestinal flora into short chain fatty acids in an *ex vivo* incubation model with fecal samples (Mallillin et al., 2008). Canine intestinal microflorae were also modifiable by dietary fiber to change short chain fatty acid profiles (Fahey et al., 2004; Middelbos et al., 2010). Diets high in fiber and protein have been shown to improve satiety (German, 2006; Yamka et al., 2006) and low glycemic index starch has been shown to alter lipid profiles (Mitsuhashi et al., 2010) in dogs during BW loss.

Rodent and human studies have shown that increased dietary bean intake inhibits tumor formation (Gupta et al., 2010), controls and manages diabetic outcomes (Venn and Mann, 2004; Villegas et al., 2008), improves blood lipids for reduced risk of cardiovascular disease (Shi et al., 2004), and enhances intestinal health (Zhou et al., 2010). Epidemiological studies indicate that legume consumption may be an important dietary predictor of longevity in humans and model organisms (Darmadi-Blackberry et al., 2004; Mensack et al., 2010; Park et al., 2011). These chronic disease-fighting properties for increasing bean consumption reported in studies with humans and rodents merit investigation in companion canines.

Dry beans may not have been incorporated into commercial canine diet formulations because of perceptions that increased cooked bean intake would result in increased flatulence and intestinal distress, or it is unpalatable to dogs. The lack of change in fecal characteristics or flatulence between the study groups was a compelling finding from this trial, given the relatively high concentration of navy beans (25%). The lack of change in flatulence from canine bean consumption supports the rationale for conducting double blinded, placebo controlled nutrition intervention studies in free-living animals.

The antinutritional components of beans may be another reason why beans have not been used in commercial canine diet formulations. Bean antinutrients are primarily found in raw, uncooked beans (Jamroz and Kubizna, 2008). Cooking reduces antinutrient amounts substantially (e.g., amylase inhibitors, trypsin inhibitors, and the lectin protein phytohemagglutinin), and use of cooked bean powder further reduces potential for deleterious effects (Rehman and Shah, 2005; Martin-Cabrejas et al., 2009). Using a commercial source of cooked navy bean powder and incorporation into a dry expanded product reduces the potential for canine antinutrient ingestion.

Considering the safety and digestibility of beans reported herein, the effects of beans for canine chronic disease control and prevention merits further exploration. We believe that the incorporation of 25% bean powder is a practical starting dose for achieving results from functional food attributes and that further digestibility

testing may be required to safely increase the quantity of beans in canine diets. It should be noted that for dogs with a high risk for urolithiasis, inclusion of legumes may be inappropriate as legumes are relatively high in purines, protein, Ca, and Mg, and may, therefore, aggravate symptoms (Hand, 2000). In conclusion, canine diets containing cooked navy bean powder are safe, digestible, and palatable, and show promise to become a novel food ingredient for dogs. This study may serve as the foundation for future canine nutritional intervention studies with cooked bean powders and for a variety of disease conditions.

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