



## Original Research

# Navy and black bean-based dog foods are digestible during weight loss in overweight and obese adult companion dogs

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## Summary

Common beans (*Phaseolus vulgaris* L.) are a nutrient-dense, low glycemic index food that supports healthy weight management in people and was examined for dogs. The objectives of this study were to evaluate the apparent total tract digestibility (ATTD) and nutrient utilisation of navy (NB) and black (BB) bean-based diets in overweight or obese companion dogs undergoing a weight loss intervention. A nutritionally complete, dry extruded dog food was used as the control (CON) diet and two iso-caloric, nutrient matched bean diets, containing either 25% w/w cooked BB or NB powder formed the test diets. Diets were fed to adult, overweight companion dogs for either four weeks (short-term study, n = 30) or for twenty-six weeks (long-term study, n = 15) at 60% of maintenance calories for ideal weight. Apparent weight loss increased over time in both the short- and long-term studies (p < 0.001) but was not different between the three study groups: apparent weight loss was between 4.05% – 6.14% for the short-term study and 14.0% – 17.9% in the long-term study. The ATTD was within expected ranges for all groups, whereby total dry matter and crude protein ATTD was 7–8% higher in the BB diet compared to CON (P < 0.05), crude fat ATTD was similar across all diets, and nitrogen free extract ATTD was 5–6% higher in both BB and NB compared to CON (P < 0.05). Metabolisable energy was similar for all diets, and ranged from 3,434–3,632 kcal/kg. At the end of each study period, dogs had haemoglobin levels ≥12 g/dl, packed cell volume ≥36%, albumin ≥2.4 g/dl, ALP ≤300 IU/l and all median values for each group were within defined limits for nutritional adequacy. This investigation demonstrated that BB and NB diets were safe, digestible, and supported weight loss in calorically restricted, overweight or obese, adult companion dogs.

**Keywords:** Dogs: weight loss: digestibility: beans: obesity: novel protein

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## Introduction

Obesity is the primary nutritional disorder in companion dogs (German, 2010). Recent surveys estimated that 34–59% of pet dogs in the United States, Europe and China are overweight or obese (Linder *et al.*, 2013; Mao *et al.*,

2013; McGreevy *et al.*, 2005). Overweight dogs can have a shorter, reduced-quality of life (German, 2010; Kealy *et al.*, 2002; Linder *et al.*, 2013) and an increased risk for developing chronic diseases such as diabetes, cardiovascular and respiratory disease, urinary tract

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infections, pancreatitis, osteoarthritis, and some types of cancer (German, 2006; Linder, 2014).

For clinically healthy dogs, the primary treatment for obesity is nutritional therapy (NRC, 2006). Because excess adiposity is directly related to a positive energy balance, the most practical dietary approach for weight loss is caloric restriction. An adequate weight loss diet has a nutrient composition that supports lean mass retention, induces fat mass reduction, and increases satiety (Linder *et al.*, 2013). Diets high in protein and fibre have been shown in both humans and dogs to promote weight loss and maintain lean muscle mass (Butterwick and Markwell, 1997; German *et al.*, 2010), as well as reduce voluntary food intake in dogs (Weber *et al.*, 2007). Emerging research has shown that, in addition to macronutrients, there are specific feedstuffs and dietary patterns that may promote weight loss as a function of bioactive components and phytochemicals (Deibert *et al.*, 2004; Rayalam *et al.*, 2008; Shai *et al.*, 2008). For example, in humans the consumption of non-soy legumes such as common beans (*Phaseolus vulgaris*, L.), split peas, lentils, and chickpeas is associated with decreased risk for obesity, (Papanikolaou and Fulgoni, 2008), reduced adiposity without caloric restriction (Mollard *et al.*, 2012), voluntary reduction of caloric intake (Borresen *et al.*, 2014), increased satiety, and in some cases, resulted in higher levels of weight loss with 30% caloric restriction compared to an isocaloric, low legume or legume-free diet intervention (McCrorry *et al.*, 2010).

Common beans, such as navy, black and pinto varieties, are excellent candidates for a weight loss-promoting food because they contain high quality protein, have a carbohydrate profile with a low glycemic index, are abundant in dietary fibre, and are rich sources of iron, zinc, folate and magnesium (Mudryj *et al.*, 2014). The high protein content and amino acid profiles of beans have been associated with increased energy expenditure during weight loss and the arginine and glutamine content in particular was associated with improved carbohydrate and fat oxidation (Rebello *et al.*, 2014). The fibre fraction from beans is abundant in resistant starch, which can augment weight loss via slower carbohydrate digestion and increased microbial fermentation (Hayat *et al.*, 2014; McCrorry *et al.*, 2010). Furthermore, bean fibre provides prebiotics sources for the gut microbiome, which contributes to energy balance via production of short chain fatty acids (SCFA) that have been shown to regulate hormones involved in food intake regulation, such as glucose-like protein 1 (GLP-1) and leptin (Huazano-Garcia *et al.*,

2015). Common beans contain a wide range of bioactive phytochemicals such as alpha-amylase inhibitors, phenolic compounds, and phytosterols which may modulate excess nutrient absorption, reduce dietary energy availability, promote satiety, and improve lipid metabolism (Barrett and Udani, 2011; Chávez-Santoscoy *et al.*, 2014; McCrorry *et al.*, 2010; Ramírez-Jiménez *et al.*, 2015). Due to the fact that dry bean consumption promoted weight loss in humans and rodents, the potential of beans to promote weight loss in dogs merits investigation because dogs have similar digestive physiology, obesity related co-morbidities and environmental exposures to people.

Common beans are safe and digestible in normal, healthy weight dogs (Forster *et al.*, 2012a). Bean-based diet formulations support short-term apparent weight loss, and were reported effective at reducing low density lipoprotein (LDL), high density lipoprotein (HDL), and triglycerides (TG) when compared to a control, bean-free diet (Forster *et al.*, 2012b). Therefore, the objectives of this current study were to: 1) evaluate the apparent total tract digestibility (ATTD) of nutritionally complete, navy (NB) and black (BB) bean diets in overweight or obese dogs undergoing calorically restricted weight loss and 2) determine the nutritional adequacy and utilisation bean-based diets compared to a bean-free, nutrient-matched control (CON) diet using the outcome measurements defined by the Association of American Feed Control Officials (AAFCO, 2010) compared to an isocaloric, nutrient matched, standard ingredient, control diet. It was hypothesised that cooked bean powders added at 25% weight/weight (w/w) into a nutritionally complete extruded dog food formulation will be digestible, support weight loss, and maintain indices of nutritional adequacy as compared to a bean-free control diet.

## Materials and Methods

### Study design

The four-week (short-term) and twenty-six-week (long-term) studies were conducted as randomised, double-blinded, controlled dietary intervention clinical trials for calorically-restricted weight loss comparing three study diets: CON, NB, or BB. The short-term weight loss study was conducted at the Colorado State University Veterinary Teaching Hospital (Fort Collins, CO, USA) and the long-term study was conducted at the Wellington Veterinary Hospital (Wellington, CO, USA). Owners signed an informed consent form and provided a medical history before dogs were enrolled in the study.

All dogs were transitioned to the study-provided diet (CON, NB, BB) over a four day period by increasing the proportion of the test diet mixed into the dog's regular food as previously described (Forster *et al.*, 2012a). At the end of the study period, all dogs were transitioned back to their regular food. Owners were instructed to exclusively feed the study kibble in the amounts prescribed. For the short-term study, owners were given pre-measured daily packets of food, and in the long-term study owners were given measuring cups with lines marked to indicate the appropriate amount of kibble to feed daily. All owners were instructed to feed only the prescribed dog food for the duration of the study. Water was provided *ad libitum* and no treats were allowed.

Body weights were assessed every two weeks and caloric intake was adjusted as needed to achieve a target weight loss of 0.5% – 2% body weight per week. For the short-term study (n = 30), a 96-hour faecal collection was performed after the dogs had been exclusively consuming the study food for 10 days. For the long-term study (n = 15) a 96-hour faecal collection was performed 12 weeks into the trial. Owners were instructed to collect faecal samples from the dogs within five hours of being voided. Samples were frozen and stored at –20° C until analysis. Compliance to the study protocol was determined by owner surveys, diet logs (short-term study only), number of faecal samples collected and apparent weight loss.

The Colorado State University Institutional Animal Care and Use Committee approved all clinical trial operations, animal care procedures, and collection of biological samples for analysis before beginning the study (IACUC 13-4316A).

Adult male and female dogs between the ages of 2–7 years, with a body condition score (BCS) of at least six on a nine point scale (Laflamme, 1997) and a body weight of at least 10 kg, with no known health concerns were recruited for study participation. All dog owners provided written informed consent for participation. After enrolment, all dogs were evaluated by the study veterinarian, assessed for haematological and biochemical anomalies, assigned a BCS, and screened for hypothyroidism with a total thyroxine (T4) test as previously described (Forster *et al.*, 2012b). Dogs were excluded from participation for hypothyroidism, abnormal blood results (unless determined by the veterinarian to be within normal limits for a specific dog), or a history or diagnosis of cancer, inflammatory disease, or current infection. Dogs were also excluded if they had been administered antibiotics or analgesics within one month

of starting the study. The preventive use of anthelmintics was allowed. Dogs could be removed from the study at the discretion of the study veterinarian or request of owner. All dogs were monitored throughout the study for adverse changes in clinical blood, serum, or plasma samples. At the end of each study period, haemoglobin, packed cell volume (PCV), albumin, and alkaline phosphatase (ALP) were compared to the AAFCO reference ranges for nutritional adequacy (AAFCO, 2010). One dog (short-term study, CON) had chronically-elevated ALP (830 g/dl at baseline that decreased to 320 g/dl at four weeks) and participated at the discretion of the attending veterinarian. Haemoglobin and PCV values were not obtained from one dog at the end of study (long-term study, BB) due to a clotted blood sample, post-collection.

Of the fifty-six dogs screened for participation in the short term or long term weight loss study, 49 were enrolled. Seven dogs failed the pre-screen exam for either renal or hepatic abnormalities (n = 2), detection of previously undiagnosed cancer (n = 2), hypothyroidism (n = 1), urinary tract infection (n = 1) or aggression and difficult handling (n = 1). Thirty-three dogs were enrolled in the short-term study and randomised based on BCS to CON, BB, or NB study groups. Three dogs were withdrawn due to physical injury (n = 1), owner unable to keep study-related appointments (n = 1) and not consuming the study provided dog food (n = 1) (Forster *et al.*, 2012b). Sixteen dogs were enrolled in the long-term study and randomised based on BCS to the CON, BB, or NB study groups. One dog was withdrawn from the long-term study after diagnosis with tapeworm and the owner's non-compliance to protocol by feeding dog treats. Individual characteristics of each dog are presented in Appendix 1 and summaries of the baseline characteristics are shown in Table 1. Breeds included dogs from retriever, terrier, herding, and working lineages, and spanned both purebred and mixed breeds. Dogs were equally distributed between study diet groups for age, weight, sex and BCS. There was one dog in each of the short-term and long-term studies that was not neutered. One eight year-old dog was included in the short-term study and one 10-year-old dog was included in the long-term study at the discretion of the study veterinarian.

### *Dietary formulations*

CON, BB, and NB diets were provided as a dry, extruded, kibbled dog food that was formulated to

**Table 1.** Baseline characteristics of dogs completing cooked bean powder-based calorically restricted weight loss study interventions

Characteristic	Control Group		Black Bean Group		Navy Bean Group		P-value <sup>4</sup>
	Median (IQR)		Median (IQR)		Median (IQR)		
Age <sup>1</sup> , yr							0.16
Short-term	6.0 (4.7–7.0)		5.0 (2.8–5.3)		4.5 (3.5–6.0)		0.10
Long-term	3.0 (2.0–8.5)		3.0 (3.0–4.5)		6.0 (4.5–7.0)		0.32
Body weight, kg							0.25
Short-term	34.5 (20.7–39.6)		28.8 (16.5–34.1)		29.25 (20.4–38.1)		0.59
Long-term	36.5 (29.2–40.4)		37.7 (26.7–45.7)		39.5 (27.3–56.1)		0.59
	Number of Dogs						
Sex <sup>2</sup>							0.61
Female							
Short-term	7	3	6	4	4	6	0.86
Long-term	3	2	4	1	2	3	0.89
BCS <sup>3</sup>							0.33
BCS 6–7							
Short-term	7	3	4	6	7	3	0.77
Long-term	2	3	2	3	1	4	0.99

Thirty dogs completed the short-term, 4 week study: Control Diet, N = 10; Black Bean Diet, N = 10; Navy Bean Diet, N = 10. Fifteen dogs completed the long-term, 6 month study: Control Diet, N = 5; Black Bean Diet, N = 5; Navy Bean Diet, N = 5.

<sup>1</sup>Age as reported by owner.

<sup>2</sup>All dogs were neutered with the exception of one female in the short-term study control group and 1 female in the long-term study black bean group.

<sup>3</sup>Body Condition Score (BCS) was determined using a 9 point scale (Laflamme, 1997).

<sup>4</sup>Continuous variables (age and weight) were evaluated for differences across groups using a Kruskal-Wallis test and categorical variables (sex and BCS) were evaluated using a Chi-square test. P values are shown across short-term and long-term studies and within study across diet. P < 0.05 was considered significant.

meet nutritional recommendations for adult dog maintenance (AAFCO, 2010; NRC, 2006) and adjusted to consist of 27% protein and 8% fat as-fed. The CON, BB, and NB diets were mixed and manufactured under the same conditions and location (ADM Alliance Nutrition Feed Research Pilot Plant, Quincy, IL; Applied Food Biotechnology Plant, St. Charles, MO) and formulated to be isocaloric and containing equivalent levels of nutrients. The CON diet ingredients consisted of poultry meal, wheat, corn, brewer's rice, pork and bone meal, flaxseed, fishmeal, brewer's yeast, and added vitamins and minerals (Table 2).

The BB and NB diets contained identical ingredients as the CON diet with the inclusion of cooked BB or NB bean powder (ADM Bean Specialties, Decatur, IL) added at 25% w/w to the BB and NB diets. To account for the inclusion of the cooked bean powders, the wheat and corn ingredients were reduced to achieve iso-nutrient formulations to the CON diet. The metabolisable energy (ME) of the diets was calculated using modified Atwater Factors and estimated at 3,314 kcal/kg (NRC, 2006).

#### Calculations for energy requirements and caloric restriction

BCS was used to estimate ideal bodyweight (BW), and determined using a nine point scale (Laflamme, 1997). A score of less than four was considered underweight, a score of either four or five was considered ideal BW, a score of six or seven was overweight, and a score of eight or nine was considered obese (Forster *et al.*,

2012b). For each BCS point over five, a dog was considered to be 10% above his or her ideal body weight in kilograms (German *et al.*, 2009). Using ideal weights determined by BCS, daily ME requirements for weight maintenance were calculated for each dog using the following formula:

$$\text{ME(kcal/day)} = 110 \times (\text{ideal BW, kg})^{0.75}$$

(Forster *et al.*, 2012a; NRC, 2006)

Dogs were calorically restricted to approximately 60% of their maintenance energy requirement.

#### Proximate analysis, apparent total tract digestibility, and bomb calorimetry

Proximate analysis was used to determine the crude nutrient profiles of the food and faecal samples as previously reported (Forster *et al.*, 2012a). Soluble and insoluble fibre fractions were determined as described by Prosky *et al.* (1992). ATTD was evaluated at two weeks for the short-term study, and at twelve weeks for the long-term study and was calculated for total dry matter (TDM), crude protein (CP), crude fat (CF), and nitrogen free extract (NFE). The following formula was used to determine NFE:

$$\text{NFE \%} = \text{TDM \%} - \text{CP \%} - \text{CF \%} - \text{crude fibre \%} - \text{ash \%}$$

For each nutrient component, the ATTD was calculated on a dry matter (DM) basis using the following formula

**Table 2.** Diet ingredient and chemical composition

Ingredient % (as-fed)	Control Diet	Black Bean Diet	Navy Bean Diet
Black bean (cooked powder)	–	25.00	–
Cooked navy bean powder	–	–	25.00
Poultry meal	19.53	19.00	19.61
Wheat grain	19.00	2.66	3.62
Wheat middlings	19.00	11.61	9.42
Corn grain	16.11	17.67	19.00
Brewer's rice	10.00	10.00	10.17
Pork and bone meal	7.32	3.95	2.56
Poultry fat	3.00	3.00	3.00
Flaxseed	1.00	1.00	1.00
Fish meal	1.00	1.00	1.00
Brewer's yeast	1.00	1.00	1.00
Digest	1.00	1.00	1.00
Calcium carbonate	0.80	1.28	1.47
Salt	0.50	0.50	0.50
Vitamin-trace mineral premix <sup>a, b, c, d</sup>	0.50	0.50	0.50
Potassium chloride	0.14	0.05	0.05
Choline chloride	0.10	0.10	0.10
Monocalcium phosphate	–	0.68	1.00
<b>Analysed Composition</b>			
<b>% (as-fed)</b>			
Dry matter	95.02	95.59	94.96
Moisture	4.98	4.41	5.04
Crude protein	26.60	26.90	26.30
Nitrogen free extract	47.82	47.99	48.96
Acid hydrolyzed fat	8.40	8.10	8.00
Crude fibre	3.90	4.30	3.70
Total dietary fibre	16.98	17.96	18.65
Soluble fibre	4.05	3.36	5.25
Insoluble fibre	12.93	14.60	13.40
Ash	8.30	8.30	8.00
Gross energy, kcal/kg	4,505	4,371	4,375
Est. metabolisable energy, kcal/kg	3,314	3,314	3,314

a. Provided per kilogram of control, black bean, and navy bean diets: 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, 1.88 mg; folic acid, 0.26 mg; vitamin B12, 37.5 µg; choline, 534.4 mg; Fe from ferrous sulfate, 282 mg; Cu from copper sulfate, 15 mg; I from calcium iodate, 2.025 mg.  
 b. Manganese from manganous oxide provided per kilogram: 10.125 mg (control, black bean), 32.01 mg (navy bean).  
 c. Zinc from zinc oxide provided per kilogram: 213.068 mg (control), 150 mg (black bean), 198.02 mg (navy bean).  
 d. Selenium from sodium selenite provided per kilogram: 0.6463 mg (control), 0.2250 mg (black bean, navy bean).

(AAFCO, 2010):

$$\text{ATTD}\% = \frac{[(\text{g of nutrient consumed} - \text{g of nutrient excreted}) / (\text{g of nutrient consumed})] \times 100.}$$

Total gross energy (GE) content was measured by bomb calorimetry for each diet and in each faecal sample taken at two weeks for the short-term study and at twelve weeks for the long-term study. ME (kcal/kg) was determined at two weeks during the short-term study, and at twelve weeks during the long-term study and reported in

kcal/kg using the following formula (AAFCO, 2010):

$$\text{ME} = \frac{\{ \text{GE consumed} - \text{GE of faeces} - [(\text{protein consumed g} - \text{protein in faeces g}) \times 1.25] \}}{\text{food consumed g} \times 1,000}$$

where GE was in kcal/g, 1.25 kcal/g was used as the correction factor for energy lost in urine, and both diet and faecal values were on a DM basis.

Dogs were excluded from the ATTD and ME analysis if owners reported dietary indiscretion during the faecal collection period, were unable to differentiate between samples from different dogs, or collected faecal samples for less than three days. CON group exclusions: short-term n = 4 and long-term n = 1; BB group exclusions: short-term n = 1 and long-term n = 1; and NB group exclusions: short-term n = 3 and long term n = 0.

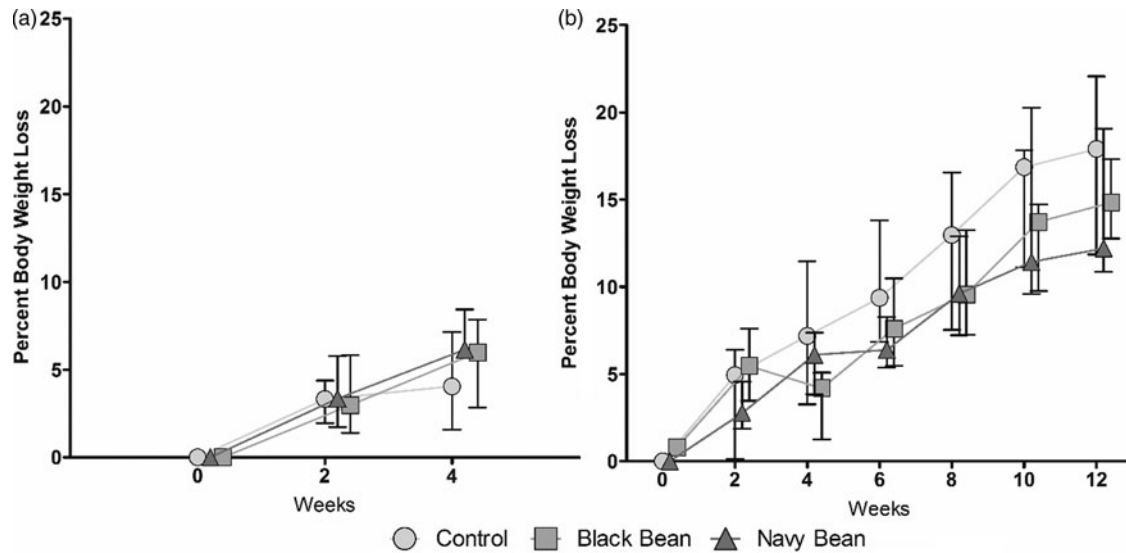
Non-parametric analyses were performed on all measures. For percent apparent weight loss, a two-way ANOVA (repeated measures) was performed within each study. For ATTD, ME, and food intake/kg BW, a two-way ANOVA (non-repeated measures) was performed. Bonferroni *post-hoc* tests were applied to correct for multiple comparisons. Statistical analyses were performed using GraphPad Prism, Version 5.03 (San Diego, CA, USA). Confidence limits were set at 95% (P < 0.05).

## Results and Discussion

### Nutrient profiles of bean-based dog foods

Proximate analysis and bomb calorimetry results confirmed that the CON, NB and BB dog food formulations were equal in nutrient levels and isocaloric (Table 2). On an as-fed basis, for all diets, the estimated ME content of the diets was 3,314 kcal/kg, CP content was approximately 26%, and CF was 8%. Crude fibre was similar between the CON, BB, and NB diets (~4%), while TDF was ~1% higher in NB and BB diets when compared to CON. Insoluble fibre was slightly increased in the BB diet (~1.5%), while soluble fibre was slightly increased in the NB diet (~1%).

There was no difference in sex, median age, or BCS between dietary treatment groups or studies (Table A1). In both the short and long-term studies, percent apparent weight loss increased over time (P < 0.0001) and was similar between dietary treatments in each study. In the short-term study the median weight loss was 4.05% in the CON group, 5.98% in BB, and 6.14% in NB. For



**Figure 1.** Percent apparent weight loss in dogs consuming a bean-based or control diet over (a) 4-weeks (short-term study,  $n=30$ ) and (b) 12-weeks (long-term study,  $n=15$ ). In both (a) and (b) percent apparent weight loss increased over time ( $p < 0.05$ ), but not between dietary treatments at any time point. Data are shown as median and IQR.

the long-term study, the median weight loss was 17.90% in CON, 14.0% in BB, and 12.21% in NB (Figure 1).

Daily nutrient intake (g/kg ideal BW) was not different between groups (Table 3) except for soluble fibre, which was significantly higher ( $P < 0.001$ ) in NB group ( $\sim 0.4$  g/kg ideal BW) compared to BB ( $\sim 0.25$  g/kg ideal BW), but not different from CON ( $\sim 0.3$  g/kg ideal BW,  $P > 0.05$ ). All dogs consumed approximately 2.5 g CP per kg ideal BW (medians ranged from 2.1 g – 2.7 g/kg ideal BW), and dogs within the BB and NB group consumed, on average, 2 g cooked bean powder per kg ideal body weight (Table 3).

#### Apparent total tract digestibility and metabolisable energy of black and navy bean-based dog diets during weight loss

In the CON, BB, and NB diets, nutrient ATTD was consistent with expected ranges for standard ingredient and bean-based extruded dog diets (Forster *et al.*, 2012a). TDM, CP, CF, and NFE ATTD are presented as median and range (min-max; Table 4). There were no differences in ATTD between each study group of the short and long-term study. In the short-term study, median TDM ATTD was higher ( $P < 0.05$ ) for BB (83.0%) than CON (74.6%), while NB was similar to both (80.2%). In the long-term study, TDM ATTD was similar for all three diets: CON (73.7%), BB (79.6%), and NB (77.5%). For the NB diet, these results were consistent with previous studies demonstrating equal TDM

ATTD compared to a nutrient matched CON diet (Forster *et al.*, 2012a). To our knowledge, this is the first report of the ATTD of a BB based canine diet. To verify that TDM ATTD was higher for the BB diet compared to CON, we performed a pooled analysis on the results from both trials. The differences in TDM ATTD between CON and BB remained significant (data not shown), further supporting that the BB TDM was indeed more digestible than CON and may have not been significant in the long-term study due to the sample size.

In the short-term study, CP ATTD was higher in BB (85.7%) compared to CON (78.6%;  $P < 0.01$ ), and NB was similar to both (83.5%). In the long-term study, CP ATTD was similar between the CON (80.9%), BB (82.5%), and NB (79.4%). Again, the difference in CP ATTD between CON and BB remained significant when data from both the long and short-term studies were pooled (data not shown). During the long-term study, the consistency of CP digestibility and cumulative weight loss between dietary treatments supported the use of common beans as a staple ingredient in weight loss dog food formulas. Past work with dogs has demonstrated that dietary protein intake is associated with lean mass retention and is central in facilitating a healthy metabolism during weight loss (Laflamme, 2012). Given that cooked beans are both highly digestible and capable of supporting weight loss, the role of bean-based diets as a novel protein source in supporting canine lean mass retention warrants further investigation.

**Table 3.** Daily nutrient intake of forty-five overweight or obese adult, companion dogs undergoing calorically restricted weight loss on nutritionally complete diets.

Daily Intake/kg ideal BW	Control Diet Median (IQR)	Black Bean Diet Median (IQR)	Navy Bean Diet Median (IQR)	P-value
Total dietary intake, g ( <i>As-fed</i> )				
<i>Short-Term</i>	9.0 (8.5–9.7)	9.5 (8.8–10.2)	8.9 (8.6–10.3)	0.939
<i>Long-Term</i>	8.8 (8.2–10.1)	8.8 (8.5–9.0)	8.7 (8.6–9.1)	
ME (Estimated) intake, kcal ( <i>DM</i> )				
<i>Short-Term</i>	1.4 (1.4–1.7)	1.6 (1.5–2.0)	1.5 (1.5–1.8)	0.510
<i>Long-Term</i>	1.3 (1.3–1.6)	1.3 (1.2–1.5)	1.5 (1.3–1.6)	
Total DM intake g/kg ideal BW				
<i>Short-Term</i>	8.7 (8.4–10.0)	9.5 (8.5–12.0)	8.5 (8.2–10.0)	0.900
<i>Long-Term</i>	8.0 (7.8–10.0)	7.4 (7.1–8.9)	8.3 (7.3–9.0)	
Crude protein, g ( <i>DM</i> )				
<i>Short-Term</i>	2.4 (2.4–2.9)	2.7 (2.4–3.3)	2.4 (2.3–2.8)	0.808
<i>Long-Term</i>	2.2 (2.2–2.8)	2.1 (2–2.5)	2.3 (2.0–2.5)	
Crude fat, g ( <i>DM</i> )				
<i>Short-Term</i>	0.8 (0.7–0.9)	0.8 (0.7–1.0)	0.7 (0.7–0.8)	0.729
<i>Long-Term</i>	0.7 (0.7–0.9)	0.6 (0.6–0.8)	0.7 (0.6–0.8)	
NFE, g ( <i>DM</i> )				
<i>Short-Term</i>	4.4 (4.2–5.2)	4.8 (4.3–5.9)	4.4 (4.2–5.2)	0.967
<i>Long-Term</i>	4.0 (3.9–5.1)	3.7 (3.6–4.5)	4.3 (3.8–4.7)	
Crude fibre, g ( <i>DM</i> )				
<i>Short-Term</i>	0.4 (0.3–0.4)	0.4 (0.4–0.5)	0.3 (0.3–0.4)	0.087
<i>Long-Term</i>	0.3 (0.3–0.4)	0.3 (0.3–0.4)	0.3 (0.3–0.4)	
TDF, g ( <i>DM</i> )				
<i>Short-Term</i>	1.4 (1.4–1.7)	1.6 (1.5–2.0)	1.5 (1.5–1.8)	0.510
<i>Long-Term</i>	1.3 (1.3–1.6)	1.3 (1.2–1.5)	1.5 (1.3–1.6)	
Soluble fibre, g ( <i>DM</i> )				
<i>Short-Term</i>	0.3 (0.3–0.4) <sup>ab</sup>	0.3 (0.3–0.4) <sup>a</sup>	0.4 (0.4–0.5) <sup>b</sup>	<0.0001
<i>Long-Term</i>	0.3 (0.3–0.4) <sup>ab</sup>	0.2 (0.2–0.3) <sup>a</sup>	0.4 (0.4–0.4) <sup>b</sup>	
Insoluble fibre, g ( <i>DM</i> )				
<i>Short-Term</i>	1.1 (1.0–1.3)	1.3 (1.2–1.6)	1.1 (1.0–1.3)	0.133
<i>Long-Term</i>	1.0 (1.0–1.2)	1.0 (1.0–1.2)	1.1 (1.0–1.1)	
Cooked bean powder, g ( <i>DM</i> )				
<i>Short-Term</i>	0 (0–0) <sup>a</sup>	2.4 (2.1–2.9) <sup>b</sup>	2.1 (2.1–2.5) <sup>b</sup>	<0.0001
<i>Long-Term</i>	0 (0–0) <sup>a</sup>	1.8 (1.8–2.2) <sup>b</sup>	2.1 (1.8–2.3) <sup>b</sup>	

To determine differences in daily intake between diets and studies, each nutrient was evaluated with 2-way ANOVA. There were no differences between studies or interactions terms. A Bonferroni post-test was used to determine the groups with significant differences. Groups not sharing the same letter superscript are significantly different from each other.

CF was equally digestible between all diets in the short-term study: CON (89.8%), BB (95.2%), and NB (92.9%) and the long-term study: CON (93.2%), BB (94.1%), and NB (89.4%). Carbohydrate ATTD, as measured by NFE, was higher in both the BB (89.3%) and NB (87.6%) diets compared to CON (83.2%) in the short-term study, and similar between all diets in the long-term study: CON (82.1%), BB (86.5%), and NB (85.1%). The NFE ATTD remained significantly higher in both bean groups when data from both the short and long-term studies were pooled (data not shown) supporting that the carbohydrates derived from the BB and NB were more digestible than those derived from corn and wheat based diets. Recent metabolomic studies have shown that metabolism of carbohydrates may be modulated in normal weight dogs consuming bean-based diets (Forster *et al.*, 2015) even though carbohydrate digestibility was the same as the a control diet (Forster *et al.*, 2012a). For dogs undergoing weight loss, BB diets

influenced relative NFE digestibility (Table 4), which may be due to differential modulation of carbohydrate metabolism compared to the CON diet. Future metabolomic investigations using samples from this study may reveal distinct carbohydrate compositions contributing to these effects in overweight and obese dogs.

ME was calculated for each group to determine the amount of energy provided by the CON, BB, and NB foods, which was similar between both the short and long-term studies and across all diets. Results are presented as a median (min-max, Table 4). The median ME for the CON diet was 3,446 kcal/kg for the short-term and 3,519 kcal/kg for the long-term study. In the BB diet, ME was 3,632 kcal/kg for the short-term and 3,507 kcal/kg for long-term study. In the NB diet, ME was 3,571 kcal/kg for the short-term and 3,434 kcal/kg for the long-term study. ME was highest in the BB short-term study (3,632 kcal/kg) and this was the only measured ME that was higher than the estimated ME

**Table 4.** Digestibility and metabolisable energy of three nutritionally complete diets fed to overweight or obese adult companion dogs undergoing calorically restricted weight loss.

Digestibility <sup>1</sup> %	Control Diet <sup>2</sup> Median (IQR)	Black Bean Diet <sup>3</sup> Median (IQR)	Navy Bean Diet <sup>4</sup> Median (IQR)	P-value <sup>5</sup>
Total dry matter				
Short-term	74.6 (67.0–80.7) <sup>a</sup>	83.0 (75.8–89.0) <sup>b</sup>	80.2 (66.8–83.5) <sup>ab</sup>	0.015
Long-term	73.7 (69.9–79.1)	79.6 (76.9–83.7)	77.5 (71.8–88.1)	
Crude protein				
Short-term	78.6 (73.1–84.6) <sup>a</sup>	85.7 (80.4–91.4) <sup>b</sup>	83.5 (78.7–87.4) <sup>ab</sup>	0.040
Long-term	80.9 (78.5–84.3)	82.5 (81.8–85.9)	79.4 (77.0–91.4)	
Crude fat				
Short-term	89.8 (88.7–92.9)	95.2 (88.5–97.7)	92.9 (77.3–96.4)	0.120
Long-term	93.2 (90.7–93.5)	94.1 (90.5–96.1)	89.4 (87.1–95.9)	
Nitrogen free extract				
Short-term	83.2 (76.9–86.8) <sup>a</sup>	89.3 (82.3–91.9) <sup>b</sup>	87.6 (84.1–89.8) <sup>b</sup>	0.002
Long-term	82.1 (77.7–85.4)	86.5 (83.9–89.5)	85.1 (83.5–91.6)	
Metabolisable energy, kcal/kg				
Short-term	3,446 (3,188–3,674)	3,632 (3,348–3,804)	3,571 (3,235–3,689)	0.617
Long-term	3,519 (3,271–3,670)	3,507 (3,367–3,618)	3,434 (3,328–3,844)	

<sup>1</sup>Digestibility was calculated on a DM basis. <sup>2–4</sup>Five, two, and three dogs were excluded from analysis in the CON, BB, and NB groups, respectively. Total number of dogs analyzed: CON, n = 6 (short-term), n = 4 (long-term); BB, n = 9 (short-term), n = 4 (long-term); NB, n = 7 (short-term), n = 5 (long-term). <sup>5</sup>To determine differences in digestibilities between diets and studies, each nutrient was evaluated with 2-way ANOVA. There were no differences between studies or interactions terms (data not shown). A Bonferroni post-test was used to determine the groups with significant differences. Groups not sharing the same letter superscript are significantly different from each other.

of 3,314 kcal/kg ( $p = 0.004$ ). These data demonstrated that the energy utilisation from bean based dog food was equivalent to standard ingredient dog food formulations in dogs undergoing calorically restricted weight loss.

#### Whole blood analyses and serum biochemistry

No negative physiological effects were observed in any measured parameter (data not shown). To demonstrate the nutritional adequacy of the dog food formulations, each dog's results were compared to AAFCO reference limits or haemoglobin, PCV, albumin, and ALP at the end of the study period. Results were presented as a median (min-max) along with the AAFCO limits for each parameter (Table 5).

The median albumin for the CON group was 3.9 g/dl and 3.9 g/dl; 3.9 g/dl and 4.0 g/dl for the BB group; and 3.9 g/dl and 4.1 g/dl for the NB group for the short and long-term studies respectively. The median ALP for the CON group was 40.5 IU/l and 41.0 IU/l; 51.0 IU/l and 31.0 IU/l in the BB group; and 27.5 IU/l and 47.0 IU/l in the NB group for the short and long-term studies, respectively. The median PCV for the CON group was 51.0% and 51.0%; 49.5% and 56.0% in the BB group; and 51.0% and 51.0% in the NB group for the short and long-term studies, respectively. The median haemoglobin for the CON group was 17.7 g/dl and 18.0 g/dl; for BB was 17.7 g/dl and 20.0 g/dl; and for NB was 17.8 g/dl and 17.9 g/dl for the short and long-term studies, respectively. Serum values fell within AAFCO established

**Table 5.** Plasma and serum biochemical analysis of three diets fed to overweight or obese adult companion dogs undergoing calorically restricted weight loss.

Parameter	Control Median (Min-Max)	Black Bean Median (Min-Max)	Navy Bean Median (Min-Max)	Reference Values (Individual)
Haemoglobin, g/dl				
Short-term	17.7 (16.2–18.4)	17.7 (16.7–19.4)	17.75 (16.0–19.2)	≥14.0 g/dl
Long-term	18.0 (16.7–19.0)	20.0 (17.1–20.4)	17.9 (15.1–19.2)	(≥12.0)
Packed cell volume, %				
Short-term	51.0 (46.0–54.0)	49.5 (48.0–55.0)	51.0 (47.0–55.0)	≥42%
Long-term	51.0 (47.0–51.0)	56.0 (50.0–59.0)	51.0 (42.0–53.0)	(≥36%)
Albumin, g/dl				
Short-term	3.9 (3.6–4.2)	3.9 (3.6–4.3)	3.9 (3.3–4.4)	≥2.8 g/dl
Long-term	3.9 (3.7–4.1)	4.0 (3.9–4.1)	4.1 (3.9–4.3)	(≥2.4)
Alkaline phosphatase, IU/l				
Short-term	40.5 (27.0–320.0)	51.0 (26.0–152.0)	27.5 (16.0–76.0)	≤150 IU/L
Long-term	41.0 (23.0–75.0)	31.0 (28.0–85.0)	47.0 (12.0–74.0)	(≤300)

Values for blood and serum samples were determined at four weeks (short-term), and twenty-six weeks (long-term). Reference values were taken from AAFCO guidelines (AAFCO, 2010) for group means and individual dogs.



reference limits, demonstrating that the NB and BB dog foods provided adequate nutrition and were safe to consume during both short and long-term weight loss. AAFCO values were applied for adult dog weight maintenance because there were no established values for dogs undergoing calorically restricted weight loss. Given that differences in canine serum samples have been reported for overweight and obese dogs compared to normal weight dogs, and that changes occur during weight loss (Forster *et al.*, 2012b; Yamka *et al.*, 2006), future studies need to determine if AAFCO reference values should be adjusted for diets targeting weight management.

Conducting weight loss and digestibility studies with companion dogs, as opposed to colony dogs, presented new challenges due to owner compliance in feeding and faecal collection and lapses in dietary discretion when feeding and collecting samples in a multiple dog household. Although this study was successful in achieving weight loss, many dogs did not achieve their ideal weight during or following completion of the long-term study. This may complicate interpretation of the results, however, these challenges emphasise the need for effective communication and perhaps an accelerated translation of canine weight loss study findings to real clinic settings for body weight management planning.

### Conclusions

In this study, it was demonstrated that nutritionally complete dog foods containing cooked bean powders were digestible by overweight or obese, adult, companion dogs undergoing short or long-term calorically restricted weight loss. The dog foods supported apparent weight loss, provided utilisable energy, and the dogs maintained indices of nutritional adequacy when compared to a bean-free control dog food. The higher NFE ATTD in both the BB and NB diets compared to CON suggested that bean based dog foods may impact canine carbohydrate metabolism. It can be concluded that cooked common beans are safe and digestible when used as a major food ingredient during canine weight loss and when fed in a nutritionally complete, extruded kibble. This provides a rationale for the continued investigation of the potential for cooked beans to improve protein, lipid, and carbohydrate metabolism, which are important for overall canine health.

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### Declaration of Interest

Bean powder was supplied by ADM Edible Bean Specialties and dog food formulations and proximate analysis were completed by ADM Alliance Nutrition labs. While these studies and analysis of results were completed at Colorado State University and should be considered free from any experimental bias, commercialisation of bean-based dog foods are in early stages of development by members of this investigative team.

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**Appendix**

**Table A1.** Baseline characteristics of individual canine study participants<sup>1</sup>.

Dog ID	Study	Diet	BCS	Weight	Age	Sex	Breed
O_C1	Short-Term	Control	6	27.4	6	F/S	Dalmatian
O_C2	Short-Term	Control	7	37.3	6	M/N	Labrador Retriever Mix
O_C3	Short-Term	Control	7	62.4	4	F/I	Saint Bernard
O_C4	Short-Term	Control	7	42.7	7	F/S	Labrador Retriever
O_C5	Short-Term	Control	7	14.7	7	M/N	Welsh Corgie
O_C6	Short-Term	Control	6	22.4	5	F/S	Australian Shepherd
O_C7	Short-Term	Control	7	15.4	3	M/N	Mixed - unknown
O_C8	Short-Term	Control	8	37.7	7	F/S	Labrador Retriever Mix
O_C9	Short-Term	Control	9	38.6	6	F/S	Golden Retriever
O_C10	Short-Term	Control	8	31.6	7	F/S	Border Collie
O_BB1	Short-Term	Black Bean	8	23.8	5	F/S	Keeshond
O_BB2	Short-Term	Black Bean	6	17.2	3	F/S	Basset Hound
O_BB3	Short-Term	Black Bean	8	40.8	7	M/N	Australian Cattle Dog
O_BB4	Short-Term	Black Bean	9	27	5	F/S	Border Collie Mix
O_BB5	Short-Term	Black Bean	7	14.2	2	F/S	Boston Terrier Mix
O_BB6	Short-Term	Black Bean	9	10.7	3	M/N	Shiz Tzu
O_BB7	Short-Term	Black Bean	7	31.8	5	F/S	Pit Bull
O_BB8	Short-Term	Black Bean	7	30.6	5	M/N	Australian Cattle Dog
O_BB9	Short-Term	Black Bean	8	32.8	6	F/S	Australian Cattle Dog
O_BB10	Short-Term	Black Bean	8	38.1	2	M/N	Australian Shepherd Mix
O_NB1	Short-Term	Navy Bean	7	32.6	6	M/N	Airdale mix
O_NB2	Short-Term	Navy Bean	7	36	2	M/N	Border Collie Mix
O_NB3	Short-Term	Navy Bean	7	17.8	4	F/S	Boston Terrier
O_NB4	Short-Term	Navy Bean	7	44.2	8	F/S	Labrador Retriever
O_NB5	Short-Term	Navy Bean	8	10	4	M/N	Dachshund
O_NB6	Short-Term	Navy Bean	9	21.2	4	M/N	Dachshund
O_NB7	Short-Term	Navy Bean	6	25.9	5	M/N	Australian shepherd
O_NB8	Short-Term	Navy Bean	7	21.2	6	F/S	Australian shepherd
O_NB9	Short-Term	Navy Bean	7	34.3	2	F/S	Boxer
O_NB10	Short-Term	Navy Bean	8	44.4	5	M/N	Karelian Bear Dog Mix
O_C11	Long-Term	Control	7	33.1	10	M/N	Cocker Spaniel
O_C12	Long-Term	Control	8	36.5	3	F/S	American Spaniel
O_C13	Long-Term	Control	8	42.2	2	M/N	German Shepherd Mix
O_C14	Long-Term	Control	7	25.2	2	F/S	Labrador Retriever Mix
O_C15	Long-Term	Control	9	38.5	7	F/S	Labrador Retriever
O_BB11	Long-Term	Black Bean	8	24.9	3	F/I	Labrador/Pit Bull Mix
O_BB12	Long-Term	Black Bean	8	44.5	3	M/N	Labrador/Pit Bull Mix
O_BB13	Long-Term	Black Bean	7	28.5	3	F/S	Pit Bull
O_BB14	Long-Term	Black Bean	7	46.8	3	F/S	Labrador Retriever
O_BB15	Long-Term	Black Bean	8	37.7	6	F/S	Labrador Retriever Mix
O_NB11	Long-Term	Navy Bean	9	16.2	7	M/N	Labrador Retriever
O_NB12	Long-Term	Navy Bean	7	63.7	3	M/N	Border Collie/Corgi Mix
O_NB13	Long-Term	Navy Bean	8	39.5	6	M/N	Golden Retriever
O_NB14	Long-Term	Navy Bean	8	38.3	6	F/S	Border Collie/New Foundland Mix
O_NB15	Long-Term	Navy Bean	9	48.5	7	F/S	Labrador Retriever

<sup>1</sup>Age, sex, and breed were reported by owners. BCS: body condition score on a 9 point scale; Weight: body weight in kg; F/I: intact female; F/S: spayed female; M/N: neutered male.