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Extruded diet macronutrient digestibility: plant-based (vegan) vs. animal-based diets in client-owned healthy adult dogs and the impact of guardian compliance during in-home trials

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Introduction: Plant-based (vegan) diets for dogs are commercially available, however, research investigating long-term nutritional adequacy of these diets is scarce. Use of client-owned animals has become increasingly popular for apparent total-tract nutrient digestibility (ATTD) studies, yet low guardian compliance with the study protocol, such as providing daily dietary intake information, is a challenge. However, the impact of low diet reporting compliance on the overall ATTD results is unknown.

Methods: Sixty-one, client-owned healthy adult dogs completed a randomized, double-blinded longitudinal study. Dogs were randomly assigned into two groups that were fed either a commercial extruded meat-based diet (MEAT, n=30) or an experimental extruded vegan diet (PLANT, n=31) for 12 weeks. At the end of the study, pet guardians performed a 72-hour total fecal collection for ATTD assessment. Pet guardians were asked to complete a food diary for the duration of the trial, however only a subset of guardians (n=35) provided this food diary at the end of the study.

Results: No evidence of an association between pet guardians providing a food diary and apparent digestibility coefficients (ADC) of crude protein (CP) (p=0.14), crude fat (EE) (p=0.72), and dry matter (DM) (p=0.68) was found. Apparent digestibility coefficients for CP (p=0.52), EE (p=0.78), and DM (p=0.43) did not differ between PLANT and MEAT. Body weight and age were found to be

associated with CP (p=0.03) and DM (p=0.01) digestibility, but no association with EE (p=0.07) digestibility was present.

Discussion: These results indicate that vegan- and animal-based diets with similar nutrient profiles can have comparable nutrient digestibility. Moreover, presence or absence of a guardian-reported food diary had no effect on the overall results of the ATTD study. Further studies investigating guardian compliance for ATTD trials are needed to develop a standardized protocol and reduce current challenges and limitations related to pet guardian's participation in digestibility trials.

KEYWORDS

alternative diets, apparent total-tract nutrient digestibility, canine, owner adherence, plant-based ingredients, vegan

Introduction

The domestication of dogs has evolved over centuries from originally being used for hunting and gathering to currently, more than 63% of guardians considering dogs to be family members (Laflamme et al., 2008; Buff et al., 2014; Dodd et al., 2018). As humanization of dogs is on the rise, it is no surprise that pet guardians pay close attention to their pet's diet with some seeking to provide a diet that more closely mimics their own. As a result, trends in companion animal nutrition often follow trends seen in human nutrition (Case, 2008; Buff et al., 2014; Dodd et al., 2020) . Around the world, the vegan or plant-based lifestyle trend has been increasing among people due to a combination of personal health, animal ethics, and environmental concerns (Laflamme et al., 2008; Santeramo et al., 2018; Knight, 2023). As more pet guardians transition to a vegan diet themselves, the trend is becoming relevant in companion animal nutrition, resulting in guardians seeking plant-based alternative pet food products (Laflamme et al., 2008; Santeramo et al., 2018; Dodd et al., 2020). Currently, extruded vegan diets for dogs that are formulated to meet or exceed canine nutrient requirements are commercially available. Although these diets are formulated to meet nutrient requirements and dogs are facultative carnivores the digestibility of plant-based materials may differ from animal-based materials. Meaning that despite being formulated to meet nutrient requirements, it is unclear how well those plant-based nutrients can be digested, absorbed, and utilized by the dogs. With interest and availability of vegan diets increasing, there is a lack of research about the long-term nutritional adequacy, including the nutrient digestibility and bioavailability, of these diets (Yamka et al., 2003; Kanakubo et al., 2015; Dodd et al., 2018). Digestibility studies in dogs have mainly focused on individual plant ingredients, which cannot be translated to represent the digestibility of an entirely vegan diet (Kendall and Holme, 1982; Bednar et al., 2000; Yamka et al., 2003). Recently a study investigating mildly cooked human-grade vegan foods has been published (Roberts et al., 2023). However, there is still a lack of research investigating digestibility of extruded vegan diets for dogs. This lack of evidence has resulted in increased concerns by pet guardians, veterinarians and veterinary nutritionists due to the risk of long-term nutrient deficiencies if an entirely plant-based diet has low digestibility.

In dogs, apparent total tract digestibility (ATTD) studies have traditionally been conducted with the use of colony animals as the study population. This allows for the trial to be conducted in a controlled manner, including precise measurement and recording of feeding quantities, complete fecal collections and appropriate handling of fecal samples (German et al., 2007; Nybroe et al., 2016; Freel et al., 2021). In the past decade, a shift to using client-owned animals in these types of studies has become popular as it allows for the trial to be conducted in a more natural environment, which reduces concerns regarding animal welfare and allows for the ability to utilize a larger and more diverse population of participants (German et al., 2007; Nybroe et al., 2016; Freel et al., 2021). Using client-owned animals is seen to increase applicability of research results to the larger pet population. However, low guardian compliance is a limitation (German et al., 2007; Nybroe et al., 2016), and there is limited research about the absence of guardian-reported food diary information on the overall results of ATTD studies.

Digestibility studies have mainly focused on the difference between plant and animal protein ingredients and have indicated that the digestibility of plant and animal sources results in no difference in protein digestibility and both have the ability to meet or exceeded nutrient requirements for dogs (Bednar et al., 2000; Yamka et al., 2003; Golder et al., 2020). This is likely due to domestication and dogs residing in closer proximity to humans, causing the digestive system of dogs and humans to have some structural and functional similarities (Coelho et al., 2018). Based on these resemblances between humans and dogs and the comparable

Abbreviations: AAFCO, Association of American Feed Control Officials; ADC, apparent digestibility coefficient; AOAC, Association of Official Analytical Chemists; ATTD, apparent total-tract nutrient digestibility; BCS, body condition score; BW, body weight; CP, crude protein; DM, dry matter; EE, crude fat (ether extract); ME, metabolizable energy; NFE, nitrogen free extract.

digestibility between single plant and animal protein ingredients, it was hypothesized that the nutrient digestibility of extruded diets including or excluding animal-based ingredients is comparable in dogs. Therefore, the primary objective of this study was to examine the ATTD of an experimental vegan diet compared to a traditional animal-based diet in healthy adult client-owned dogs. It was also hypothesized that ATTD results are affected by low guardian compliance to submit food diary information during in-house digestibility studies. Hence, a secondary objective was to investigate the impact of the presence or absence of guardian-reported food diary information on ATTD.

Materials and methods

All experimental procedures for this study were approved by the University of Guelph Animal Care Committee (AUP#4192) and the Research Ethics Board (Research Ethics Approval number 19-02-036), and were in accordance with institutional, provincial, and national guidelines for the care and use of animals and humans participating in research.

Animals and experimental design

The present study was conducted as part of a larger, randomized, double-blinded longitudinal study in client-owned healthy adult dogs, which occurred between July 2019 and November 2020 (Dodd et al., 2023). Recruitment of trial participants was conducted through an eSurvey designed on the Qualtrics (Provo, Utah, USA) platform to collect data regarding eligibility for study enrollment. This survey was advertised locally around the University of Guelph campus and surrounding community, as well as shared virtually on social media to local dog-related groups. Dogs were excluded if they were reproductively intact, had a body weight (BW) less than 5kg, had an owner-reported body condition score (BCS) greater than 5 on a 9-point scale (WSAVA scoring chart, 2020), fed a homemade or raw pet food, housed outdoors without supervision, had access to unmonitored food sources, had current medical problems, or had any known dietary allergies. Dogs in households without children or other animals were prioritized. Recruitment resulted in a total of 87 dogs scheduled for enrollment appointments (Figure 1).

The enrollment appointment included discussion of the study procedures, collection of signed informed consent from pet guardians and a wellness examination of the dogs conducted by a licensed veterinarian. The wellness examination involved a medical and dietary history, a physical examination, and BW measurement. Blood was collected for complete blood count and serum biochemistry profile. Dogs were approved for inclusion of the trial if they were confirmed to be spayed/neutered, had a BCS between 4 and 7 on a 9-point-scale (Laflamme, 1997), and deemed healthy based on a physical examination and routine blood work. Seventy-six dogs met the inclusion criteria and started the 4-week adaption period during which all dogs received the same commercial extruded animal-based diet (MEAT) (Figure 1). Eleven dogs did not continue the study after the adaptation (Figure 1) due to not eating the diet, gastro-intestinal issues (vomiting or diarrhea), excessive weight gain or COVID-19 related pet guardian concerns to continue participation.

Next, the remaining 65 dogs were randomly assigned into two diet groups (Figure 1): continuing with the animal-based diet (MEAT, n=31); or being fed an experimental extruded vegan diet (PLANT, n=34). Diets were fed for 12 weeks, maintaining current energy intake, as determined based on diet history information. Four dogs were excluded during the experimental period due to pet guardian personal reasons or dog health concerns such as development of gastro-intestinal ulcers after administration of non-steroidal anti-inflammatory drugs and development of a urinary tract infection (Figure 1). Fecal collections for ATTD occurred after 12 weeks of exclusively feeding either the MEAT or PLANT diet.

Due to the COVID-19 pandemic, and public health-related restrictions on research involving human participants, the trial was paused for 4 months from March 2020 until July 2020. During this period dogs were maintained on the experimental diets, either PLANT or MEAT depending on which phase of the trial they were in (adaptation or experimental period), to allow for immediate resumption of data collection when restrictions were lifted, and the study was allowed to resume. This resulted in some variation in trial duration for dogs participating in the study, with the adaptation period for five dogs lasting more than 4 weeks (PLANT n= 2; MEAT n=3), the experimental period for two dogs lasting more than 12 weeks (PLANT n= 1; MEAT n=1), and for three dogs both the adaption period and the experimental period lasting more than 4 and 12 weeks, respectively (PLANT n=2; MEAT n=1). Examination of labelled scatter plots revealed these dogs were not identified as outliers or had results significantly different from the dogs consuming the experimental diet for the intended 12 weeks.

Diets

Both diets, MEAT and PLANT, used in this study were formulated to meet or exceed nutrient recommendations according to the AAFCO 2019 nutrient profile for canine adult maintenance (Table 1). The MEAT diet used in this study was a commercial extruded dog food (Petcurean Go! Skin + Coat Care Chicken Recipe, PPN Ltd., Chilliwack, BC, Canada). The PLANT diet was an experimental extruded dog food, not including any animal-based ingredients, formulated to be isoenergetic and as similar as possible in macronutrient and micronutrient profiles to the MEAT diet (Table 1). Pet guardians and researchers were blinded to the identity of the diets being fed to study participants throughout the duration of the testing period and remained unknown until after all data was analyzed.

Food quantity was calculated based on each dog's current dietary intake to match calories and maintain current BW and a

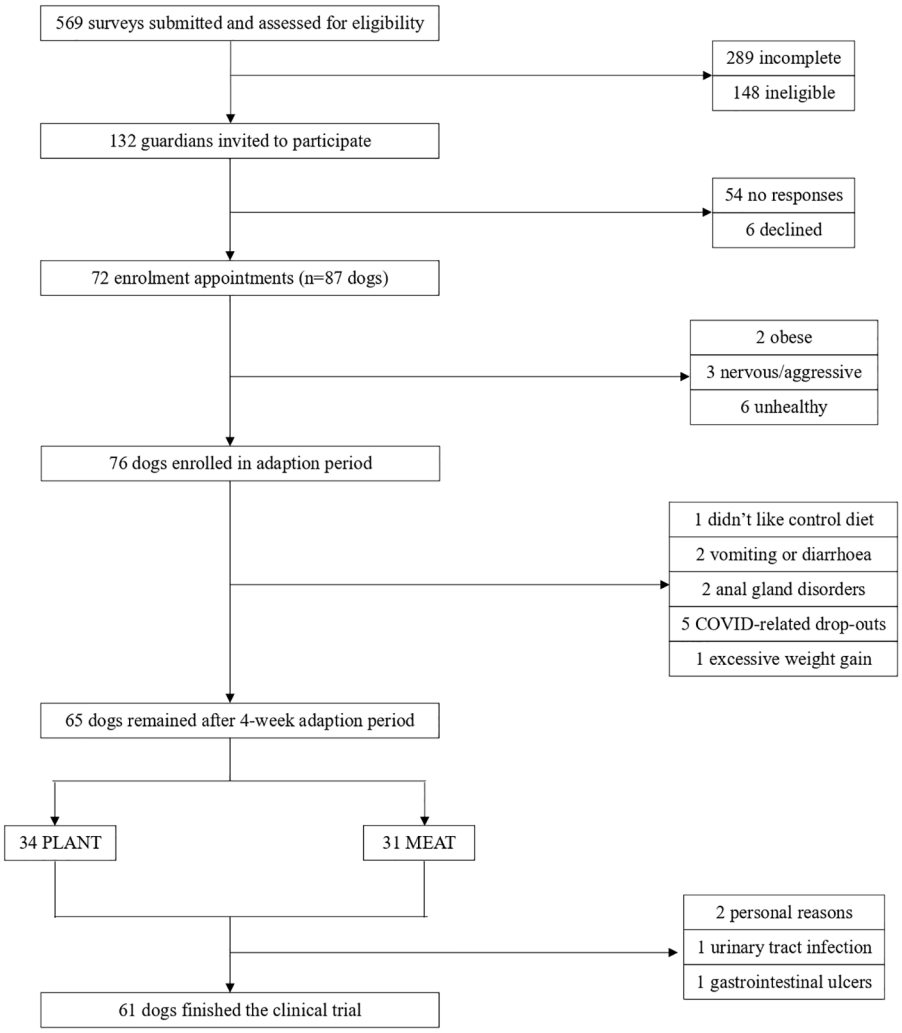

FIGURE 1
Flow chart of recruitment and enrollment of healthy adult client-owned dogs in a randomized, double-blinded longitudinal study investigation apparent total-tract nutrient digestibility of crude protein, crude fat and dry matter of an experimental vegan (PLANT) versus a commercial animal-based (MEAT) extruded diet.

gram scale was provided to each household to precisely measure and serve the recommended quantity of food per day. To help promote compliance with the study protocol, pet guardians were given a list of plant-based treats without added micronutrients, and an acceptable treat allowance was calculated for each dog to avoid exceeding 10% of their daily energy intake from sources other than the experimental PLANT or MEAT diet (Yaissle et al., 2004; Laflamme, 2006). Pet guardians were instructed not to feed their dogs any other food items as well as to record food and treat intake in a daily food diary for the duration of the study. The food diary consisted of a spreadsheet and included a section for daily morning and night diet history information such as kibble offered in grams, kibble eaten in grams, and treats consumed. Information regarding fecal score (Moxham, 2001), defecation frequency, BCS (Freeman et al., 2011; WSAVA, 2013), BW, activity (walk, dog park, etc.), and any events that occurred which could have influenced trial protocol such as but not limited to visiting another household or changes to the dog's typical routine were also included (Online Supplementary Material Table 1). Guardians were asked to return the food diary to the researchers on the day of the exit assessment.

Fecal sample collection

The ATTD study involved a 72-hour total fecal collection (Hagen-Plantinga et al., 2014) conducted by the pet guardians prior to their exit assessment appointment after 12 weeks of exclusively feeding the PLANT or MEAT diet. Fecal samples were collected immediately after voiding, frozen and delivered to the research team in a provided container stored in a Styrofoam cooler box. Samples were weighed and kept in a -20°C freezer until shipped for analysis. Samples were shipped on dry ice in a Styrofoam cooler.

TABLE 1 Nutrient profile on dry matter basis and ingredient list of the experimental vegan (PLANT) and commercial animal-based (MEAT) extruded diets fed to the client-owned dogs in this randomized, double-blinded longitudinal study.

Nutrient (g/100g DM)	PLANT[1]	MEAT[2]
Moisture	6.80	5.90
CP[3]	23.68	27.74
EE[4]	14.90	13.20
CF[5]	3.90	3.40
CA[6]	7.10	8.10
*NFE[7]	43.92	41.66
**ME[8] (g/100kcal)	419	410
PLANT Diet Ingredients		
Peas, barley, oats, potato protein, sunflower oil (preserved with mixed tocopherols), pea protein, lentils, quinoa, calcium carbonate, dicalcium phosphate, primary dried yeast, flaxseed, natural vegetable flavouring, salt, dried marine algae, choline chloride, vitamins (vitamin A supplement, vitamin D2 supplement, vitamin E supplement, niacin, L-ascorbyl-2-polyphosphate (a source of vitamin C), d-calcium pantothenate, thiamine mononitrate, riboflavin, pyridoxine hydrochloride, folic acid, biotin, vitamin B12 supplement), minerals (zinc proteinate, iron proteinate, copper proteinate, zinc oxide, manganese proteinate, copper sulphate, ferrous sulphate, calcium iodate, manganous oxide, selenium yeast), DL-methionine, potassium chloride, L-lysine, taurine, L-carnitine, dried rosemary		
MEAT Diet Ingredients		
Chicken meal, de-boned chicken, whole brown rice, white rice, oatmeal, chicken fat (preserved with mixed tocopherols), potatoes, salmon meal, natural chicken flavour, whole dried egg, flaxseed, pea fibre, alfalfa, apples, carrots, cranberries, sodium chloride, potassium chloride, dried chicory root, dried Lactobacillus acidophilus fermentation product, dried Enterococcus faecium fermentation product, vitamins (vitamin A supplement, vitamin D3 supplement, vitamin E supplement, niacin, L-ascorbyl-2-polyphosphate (a source of vitamin C), d-calcium pantothenate, thiamine mononitrate, beta-carotene, riboflavin, pyridoxine hydrochloride, folic acid, biotin, vitamin B12 supplement), minerals (zinc proteinate, iron proteinate, copper proteinate, zinc oxide, manganese proteinate, copper sulphate, ferrous sulphate, calcium iodate, manganous oxide, selenium yeast), DL-methionine, L-lysine, taurine, yucca schidigera extract, dried rosemary.		

[1]PLANT, experimental plant-based (vegan) diet.
[2]MEAT, animal-based diet.
[3]CP, crude protein.
[4]EE, crude fat.
[5]CF, crude fiber.
[6]CA, crude ash.
[7]*NFE is calculated as: 100 - CP - EE - CF - CA (AAFCO, 2019).
[8]**ME (Kcal/kg) is calculated as: 10 × [(3.5 × CP) + (8.5 × EE) + (3.5 × NFE)] (AFFCO, 2018). Both diets were formulated to be isoenergetic, isonitrogenous, and as similar as possible in nutrient profiles.

Analytical methods

Proximate analyses of the diets, including moisture, crude protein (CP), crude fat (ether extract, EE), crude fiber and crude ash, were performed at a commercial laboratory (Bureau Veritas, Mississauga, Ontario, Canada) according to Association of Official Analytical Chemists (AOAC) International's Official Methods of Analysis (AOAC, 1990; AOAC, 2005). Similarly, fecal samples were analyzed for moisture, CP, crude ash and EE at Central Testing Laboratory Ltd., Winnipeg, MB, Canada. The total 72-hour fecal collection wet samples were mixed thoroughly, and a representative subsample was taken for each analysis. All chemical analyses of feces were determined by AOAC methodology (AOAC, 1990; AOAC, 1996; AOAC, 2005).

Moisture and dry matter

Moisture and dry matter (DM) of diets and fecal samples were measured using AOAC 930.15. Moisture was analyzed by taking a subsample of approximately 100g in an aluminum pan and drying first at 100°C for a minimum of 6h. Samples were then weighed back for moisture as is values and grinded. Approximately 2g of sample was weighed and added to aluminum dish and inserted into a preheated oven of 135°C for 2h. Next, samples were weighed again and calculated for loss due to drying to obtain the moisture results. DM was calculated by 100 – moisture %.

Crude protein

Dietary and fecal CP content were measured using AOAC 992.15 and AOAC 990.03. Subsamples of diets and total fecal collections were finely ground and weighed (50 to 150mg) into tinfoil capsules. Analysis was done following the standard Kjeldahl method, meaning CP content was estimated as nitrogen multiplied by 6.25 (ISO, 2005). Nitrogen amount was determined by combustion at high temperatures (900°C– 1200°C) in an oxygen-rich environment. All nitrogen was converted to nitrogen oxide and reduced to nitrogen gas by redactor tubes. Carbon dioxide was used as a carrier gas and the elemental nitrogen was measured with a thermal conductivity detector.

Crude fat

Both diets and individual fecal samples were analyzed for EE as a measure of crude fat using AOAC 996.06 and AOCS AM 5-04. Ether extract was extracted using filter bag technology, this testing method determines fat (%) by following the Soxhlet Method (Osborne and Voogt, 1978). The Soxhlet principal method is conducted in a closed stainless-steel extraction vessel allowing solvent (90 mL of petroleum spirit) temperatures to exceed boiling points. Samples were encapsulated in XT4 filter bags and placed in a specially designed siphoning carrier that was secured in the extraction vessel. The extraction vessel was then placed over an electric heating mantel where the solvent was brought to a boil. The heat source was adjusted so the solvent drips from the condenser unit of the extraction vessel into the sample chamber at a rate of about 6 drops per second. The extraction continued for up to 6 hours, after which the vessel was removed from the heat source and the extractor and condenser units were detached from the sample chamber. The sample chamber was then placed in an oven at 102°C and dried until constant weight was reached. Dried samples were reweighed, and EE content was determined by loss of weight.

Crude fiber

Dietary crude fiber content was measured by AOCS Ba 6a-05 for diet. Samples were prepared and ground to a uniform fineness

(e.g., Retch 0.5mm). One-gram samples were sealed in F57 filter bags and pre-extracted in a beaker with ether. Up to 24 pre-extracted samples were placed in a bag suspender and processed simultaneously in the Fiber Analyzer (ANKOM^{200}, ANKOM Technology, Macedon, NY).

Crude ash

Dietary crude ash content was determined using AOAC 923.03. Samples were grounded and weighted in crucibles. The weighed crucibles were then placed in a furnace at 600°C for 2h. Samples were then cooled down to ambient temperatures and results were calculated.

Calculations

Apparent digestibility coefficients

Apparent digestibility coefficients (ADC) for CP, EE, and DM were calculated using Microsoft Excel (2018) using the following formula:

$$\text{ADC}\,(\%) = \left(\frac{(\text{amount of nutrient in the diet} \cdot 3 - \text{day feed intake}) - (\text{amount of nutrient in the feces} \cdot 3 - \text{day fecal output})}{(\text{amount of nutrient in the diet} \cdot 3 - \text{day feed intake})}\right) \times 100$$

Nitrogen free extract

Carbohydrate content in the diet was approximated using NFE and was calculated by the equation (AAFCO, 2019):

$$NFE\ (\%) = 100 - Moisture - CP - EE - Crude\ Fibre - Crude\ Ash$$

Metabolizable energy

Metabolizable energy (ME) of the diet was calculated as kilocalories per kg as fed (kcal/kg), using the equation (AAFCO, 2019):

$$ME = 10\ x\ [(3.5xCP) + (8.5xEE) + (3.5xNFE)]$$

Statistical analysis

Apparent digestibility data was analyzed using R (R Core Team, Vienna, Austria). Figures were produced using the package ggplot2 (R Core Team, Vienna, Austria). Independent variables (diet, age and BW) along with dependent variables (ADC for CP, EE, DM) were tested for normality using a Shapiro-Wilk test and visual evaluation of frequency histograms and box plots. All data presented to be normally distributed. To evaluate the association of ADC with the availability of owner-reported food diary information, a linear regression model controlling for presence or absence of a food diary was performed. To evaluate the association of ADC with diet, BW, and age a secondary linear regression model controlling for BW, age, and diet was performed, not accounting for availability of the food diary. In this analysis, diet, BW, and age were included as covariates to assess their association with ADC. A p-value of <0.05 was considered statistically significant and results were reported as means ± SD.

Results

All 61 dogs remained in good health throughout the study. However, sample size for ATTD analysis was reduced to 29 dogs (PLANT n=15; MEAT n=14) for statistical analysis due to absence of fecal samples and/or untrustworthiness of participants, such as not attending check-ins or providing reports of additional treats that exceeded 10% of total daily calories throughout the trial (Online Supplementary Material Table 2). Also, two dogs were considered significant outliers and were therefore not included in the final statistical model. One dog in the PLANT group for EE digestibility and one dog in the MEAT group for DM digestibility were considered significant outliers and were therefore not included in the final statistical model for EE and DM, respectively. Of the 29 remaining dogs, six were considered small sized dogs (<14kg), 10 were considered medium sized dogs (14 – 24kg), and 11 were considered large sized dogs (>25kg). Completed food diaries were submitted by the pet guardian for 18 of the dogs (PLANT n=9; MEAT n=9), and 11 dogs had trustworthy pet guardians, based on interactions with researchers during the trial to assume total feed consumption, but who did not provide a completed food diary (PLANT n=6; MEAT n=5).

The linear regression model controlling for presence or absence of food diary information did not find evidence of an association between pet guardians providing a food diary and ADC of CP (p=0.14), EE (p=0.72) and DM (p=0.68) (Table 2). With no association found between presence or absence of a food diary, all dogs were included in the secondary linear regression model controlling for diet, BW, and age. This linear regression model found no evidence of an association on ADC of CP (p=0.52), EE (p=0.78) and DM (p=0.43) between the dogs fed the PLANT diet and the dogs consuming the MEAT diet (Table 3 and Figure 2). Body weight and age were associated with CP (p=0.03) and DM (p=0.01) digestibility, but no association with EE (p=0.07) digestibility was present (Table 3). This indicated a trend that in larger dogs as age increased, nutrient digestibility increased; while in smaller dogs nutrient digestibility decreased as age increased, and in medium-sized dogs nutrient digestibility remained constant as age increased (Figure 3).

Discussion

The results from this current study demonstrated no differences in ATTD of CP, EE and DM between the PLANT and MEAT diets, providing evidence that dogs have the ability to digest appropriately processed plant-based material to meet nutrient requirements. This demonstrates that the digestibility of some plant-based material in dogs may be comparable to the digestibility of some animal-based material. Commercial diets for dogs typically contain plant-based ingredients for starch and fiber, but also contain animal-based ingredients as the main sources of protein and fat (Kendall and Holme, 1982; Bednar et al, 2000; Yamka et al., 2003; Kamboj and Nanda, 2018). High meat commercial diets appeal to some pet guardians based on the ideology that domesticated dogs should consume a diet that closely replicates that of their carnivorous wolf

TABLE 2 Linear regression models of crude protein, crude fat, and dry matter controlling for weight, age, and food diary in 29 healthy client-owned adult dogs fed an experimental vegan (PLANT; n=18) or commercial animal-based (MEAT; n=11) extruded diet in a 12-week randomized, double blinded longitudinal study.

ADC[1]	PLANT[2]+MEAT[3] Food Diary	PLANT[2]+MEAT[3] No Food Diary	t-value	P-value
CP[4]	82.7 ± 9.6	86.6 ± 5.6	1.56	0.14
EE[5]	96.6 ± 2.3	97.5 ± 1.5	0.40	0.72
DM[6]	78.2 ± 10.3	82.0 ± 8.9	0.42	0.68

[1]ADC, apparent digestibility coefficient.
[2] PLANT, experimental plant-based (vegan) diet.
[3]MEAT, animal-based diet.
[4]CP, crude protein.
[5]EE, ether extract (crude fat).
[6]DM, dry matter.
[a]Coefficient of correlation significant at P<0.05.
No association between ADC and the availability of a food diary was found. The t-values and p-values were taken from the linear regression models, and a p-value of <0.05 was considered statistically significant.

ancestors Hare et al., 2012; (Axelsson et al., 2013; Buff et al., 2014). Although wolves may choose to eat some vegetation, the quantity of plant materials they typically consume is far less than the amount of animal tissue they consume and for that they can be considered to remain true carnivores (Zlatanova et al., 2014; Lyu et al., 2018). However, in domesticated dogs, genes for digestion and metabolism have shown coevolutionary trends with humans resulting in increased capacity for digesting and utilizing plant-derived nutrients, with dogs now being facultative carnivores rather than true carnivores like their wolf ancestors (Axelsson et al., 2013; Buff et al., 2014; Fortes et al., 2010; Hare et al., 2012; Lyu et al., 2018; Twomey et al., 2002; Wang et al., 2013).

Unlike wild wolves, domesticated dogs have a more diverse diet that includes including starch, fat and protein ingredients and can meet their nutrient requirements from a diet that is not entirely animal-based (Lyu et al., 2018). Plant-based ingredients have similar digestibility to animal-based ingredients and could be safe alternatives for use in conventional diet formulations for dogs (Bednar et al., 2000; Bednar et al., 2001; Golder et al., 2020). Bednar et al. (2000) compared a plant-based protein source (soybean meal) to animal-based protein sources (beef and bone meal, poultry by-product meal, and poultry meal) in grain-based extruded diets and demonstrated that both plant and animal protein sources had similar crude protein ATTD, suggesting soybean meal could be a good protein source suitable for use in dog foods. Similar results were demonstrated in a study by Golder et al. (2020) in which individual plant and animal protein sources were shown to have comparable protein digestibility potential. In recent research using precision-fed cecectomized and conventional rooster assays, mildly cooked human-grade plant-based foods for dogs shown to be well digested in dogs (Roberts et al., 2023). However, until this publication, research investigating ATTD has mainly focused on individual plant-based ingredients. Although these studies demonstrated some common plant-based ingredients to have high ATTD when used in dog food formulations, these results do not represent the ATTD of an entirely plant-based extruded diet (Bednar et al., 2000; Golder et al., 2020). To the authors' knowledge, the present study is the first to assess if an entirely plant-based extruded dry kibble diet has similar ATTD as a conventional animal-based diet to meet or exceed nutrient requirements of dogs. The present study found no difference between the plant-based and animal-based diets for ATTD of CP,

TABLE 3 Linear regression models of crude protein, crude fat, and dry matter controlling for diet, body weight, and age in 29 healthy adult client-owned dogs fed an experimental vegan (PLANT; CP & DM n=15, EE n=14) or commercial animal-based (MEAT; CP & DM n=14, EE n=13) extruded diet in a 12-week randomized, double-blinded longitudinal study.

ADC[1]	PLANT[2]	MEAT[2]	Differences[7]	t-value Diet	P-value Diet	t-value BW[8]: Age	P-value BW[8]: Age
CP[4]	85.0 ± 7.1	85.6 ± 6.2	0.6	0.65	0.52	2.30	0.03[a]
EE[5]	97.1 ± 2.0	97.3 ± 1.3	0.25	0.29	0.78	1.93	0.07
DM[6]	80.6 ± 10.2	80.5 ± 8.4	-0.12	0.80	0.43	2.90	0.01[a]

[1]ADC, apparent digestibility coefficient.
[2]PLANT, experimental plant-based (vegan) diet.
[3]MEAT, animal-based diet.
[4]CP, crude protein.
[5]EE, ether extract (crude fat).
[6]DM, dry matter.
[7]Differences, MEAT – PLANT.
[8]BW, body weight.
[a]Coefficient of correlation significant at P<0.05.
The correlation between MEAT and PLANT diet was not significant, associations between BW and age on digestibility were found. The t-values and p-values were taken from the linear regression models, and a p-value of<0.05 was considered statistically significant.

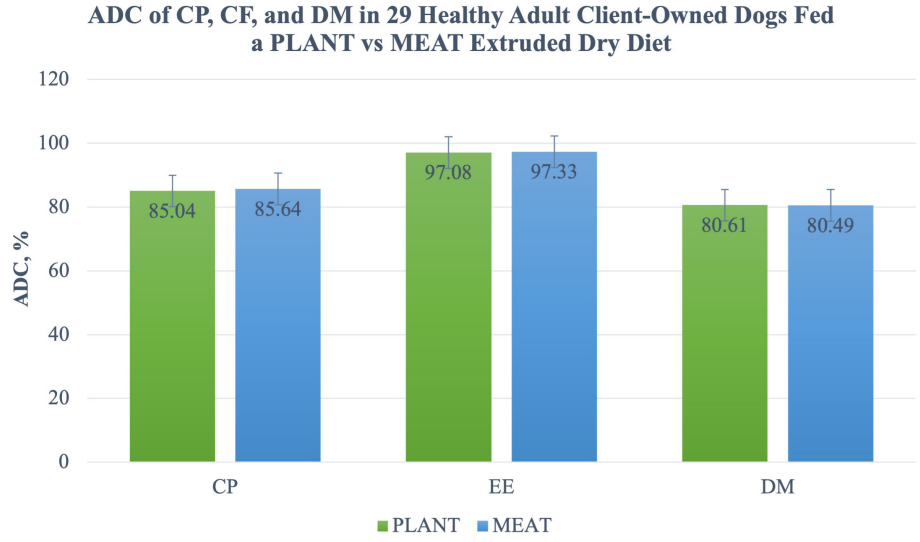

FIGURE 2
Apparent digestibility coefficient (ADC, %) of crude protein (CP), crude fat (EE, ether extract), and dry matter (DM) in 29 healthy adult client-owned dogs fed an experimental vegan (PLANT) or a commercial animal-based (MEAT) extruded diet in a 12-week randomized, double-blinded longitudinal study. (n=15 consuming PLANT for CP and DM but n=14 for EE and n=14 consuming MEAT for CP and EE but n=13 for DM). Apparent digestibility coefficients of CP, EE, and DM did not differ between diets (P>0.05).

EE and DM. These results are in line with our hypothesis that entirely plant-based diets have comparable nutrient digestibility to conventional animal-based diets, and therefore the potential to meet or exceed protein nutrient requirements if fed long-term. It should, however, be noted that the experimental plant-based diet used in the present study was formulated to be as close in ingredients and nutrient profile as possible to an existing commercial diet, and as comparable as possible to the animal-based extruded diet used in the study.

In humans, the nutrient profiles between an omnivorous diet and a plant-based diet are typically very different, with a vegan lifestyle showing to coincide with lower total energy and CP intake but increased EE and CF intake when compared to an omnivorous diet (Clarys et al., 2014). Purported health claims are likely to be associated at least in part to the differences in nutrient intake as well as the sources of those nutrients. Furthermore, in human diets, the difference in ATTD potential between an omnivorous and vegan diet may result from the different nutrient profiles of the whole diet, differences in food volume consumed and energy intake, as well as different cooking processes and diet preparations. In comparison, commercial diets designed to meet industry guidelines for dogs are specifically formulated to meet or exceed standard nutrient recommendations for the intended life stage of the animal, regardless of ingredients selected, so there the nutrient profile of a plant-based or animal-based diet is likely to be similar in essential nutrient content. Furthermore, the preparation and processing of both conventional and plant-based commercial diets for dogs are key factors in the ability to provide nutrients to the animal. Extruded kibble diets are exposed to extensive cooking processes that impact nutrient digestibility and bioavailability, and can reduce anti-nutritive factors in plant-based ingredients (Thompson, 2008; Tran et al., 2008; Tanprasertsuk et al., 2021). Therefore, the results

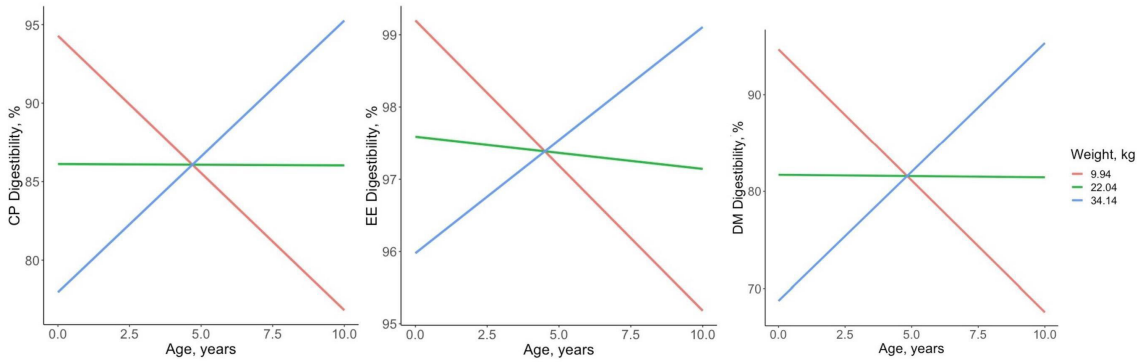

FIGURE 3
Relationship between body weight, age and digestibility from the linear regression model of apparent (CP (p=0.03), EE (p=0.07), and DM (p=0.01) digestibility controlling for BW and age in 29 healthy adult client-owned dogs fed an experimental vegan (PLANT) or commercial meat- based diet in a 12-week randomized, double blinded longitudinal study (n=15 consuming PLANT and n= 14 consuming MEAT).

of the present study show that if a plant-based diet is formulated to meet or exceed nutrient recommendations for adult maintenance and exposed to similar processing methods as conventional animal-based pet foods, it may be a suitable alternative diet for dogs.

In the present study, BW and age were found to be associated with CP (p=0.03) and DM (p=0.01) digestibility. In previous research similar results have been reported indicating that adult and large dogs show higher digestive efficiencies than puppies and smaller dogs (Weber et al., 2002). These results similarly align with findings from another study conducted in female colony dogs of various breeds, showing significant effects of age and body size on apparent digestibility (Weber et al., 2002). Our results demonstrated that as age increased in the larger dogs, ATTD of CP and DM increased as well. Body weight and age-related changes on ADC is not well understood in dogs, and this relationship requires further research to better understand our observations.

Trends in companion animal nutrition are expected to continue to closely mimic trends seen in human nutrition, resulting in new ingredients being introduced for the formulation of pet food diets (Dodd et al., 2018). As more pet guardians seek alternative feeding methods, research on ATTD to test the nutritional value and safety of new diets is becoming increasingly important (Alvarenga et al., 2019). Using client-owned animals for digestibility trials has become popular in research due to the ability for a more natural study environment and larger study population, which helps increase the applicability of the results (German et al., 2007; Nybroe et al., 2016; Herstad et al., 2017; Freel et al., 2021). However, client-owned animals present challenges to the researcher, as there is limited oversight during the trial to reduce confounding factors such as additional food consumption or inaccurate record keeping (German et al., 2007; Nybroe et al., 2016; Herstad et al., 2017; Freel et al., 2021). Using client-owned animals can be seen as both a strength and a limitation depending on the level of compliance from pet guardians since they are responsible for gathering a large portion of the data, such as food intake, fecal collection, and sample storage (German et al., 2007; van der Kooij et al., 2014; Nybroe et al., 2016; Herstad et al., 2017; Freel et al., 2021). Publications reporting in-home digestibility trials commonly describe study design limitations due to inadequate food diaries, fecal samples, and/or supervision throughout the trial (German et al., 2007; Herstad et al., 2017; Freel et al., 2021; van der Kooij et al., 2014; Nybroe et al., 2016). Similar limitations were present during this current study, as many pet guardians failed to provide either a total fecal collection or food diary at the end of the trial. Guardian compliance continues to be a large limitation to in-home digestibility trials; and yet, methodology protocols to reduce this limitation are not well investigated (Freel et al., 2021). Typical protocols for in-home ATTD trials follow similar methodology as trials using laboratory animals which are in an environment where it is easy to control food intake, fecal collection, and provide detailed feeding records (AAFCO, 2019; Freel et al., 2021). With client-owned participants, changes to the requirements for keeping daily food diaries may help to improve guardian compliance. Feeding records during digestibility trials is required to know how much food each animal consumed (German et al., 2007; Nybroe et al., 2016; AAFCO, 2019; Freel et al., 2021). Maintaining these types of records appears to be a challenge for pet guardians to complete. In the current study no association was found between the presence of a food diary and ATTD of CP, EE, and DM in either diet type. This suggests that despite failing to maintain an adequate record of dietary intake, it was likely that participants did indeed adhere to the study protocol regarding food allowance for their dogs. In traditional digestibility trials using laboratory dogs, a sample size between 5 to10 participants is typically used (German et al., 2007; Herstad et al., 2017; Freel et al., 2021; van der Kooij et al., 2014; Nybroe et al., 2016). A limited sample size can affect the accuracy of the results since it becomes difficult to determine if a particular outcome is a true finding (Röhrig et al., 2010; Button et al., 2013; Faber and Fonseca, 2014). Low guardian compliance to provide feeding records usually results in the removal of the participant to reduce the potential risk of inaccuracies, which further reduces the sample size and statistical power, and increases the risk of false positives (Nybroe et al., 2016; Herstad et al., 2017). The findings in the present trial failed to identify an effect of the availability of a food diary on ATTD which allowed for a larger sample size than if participants without a food diary had been excluded. For example, in the current study, out of the 29 dogs, only 18 dogs had food diaries provided by the owners (n=9 PLANT, n=9 MEAT). In previous at-home ATTD studies, the 11 dogs without provided food diaries would have been removed from the statistical analysis. However, the results in our current study demonstrated no association between presence or absence of food diaries, which allowed for the sample size to remain at 29 (n=15 PLANT, n=14 MEAT) (Dichev and Skinner, 2002; Borm et al., 2007).

Although low guardian compliance is a reoccurring problem in trials where dogs are evaluated in their home setting, the current trial may have had lower guardian compliance due to the ATTD trial being at the end of a longer study (Dodd et al., 2023), thus, pet guardians needed to adhere to study protocols for longer than a typical ATTD trial. Pet guardians were asked to complete a food log every day for the duration of this 3-month trial, which resulted in 38% (11/29) of pet guardians either not supplying a diary or submitting an incomplete diary to the research team. Another digestibility trial stated similar reasons as the cause for removing a participant from the trial prior to analysis (Nybroe et al., 2016). Most digestibility trials follow a 48 to 72-hour total fecal collection method (Gilberto et al., 2002; Murphy et al., 2008; Nybroe et al., 2016). Furthermore, providing the pet guardian with clear feeding instructions at the beginning and throughout the trial, as was done in the present study, may be crucial to increasing guardian compliance with the study protocol.

Fecal sample collection may have also affected the study results. Pet guardians were provided with a small Styrofoam cooler and waste collection bags and asked to collect feces immediately after defecation and place in a freezer. Despite clear instructions to freeze fecal samples immediately after defecation, there was no direct supervision by the research team to ensure that fecal collection and sample storage were performed properly by pet guardians. At this time, there is no published research examining the effects of fecal collection and storage conditions on sample quality for ATTD analysis. Therefore, there is a need to investigate the potential effects of fecal collection and storage conditions to generate a better

understanding about whether this could impact ATTD analysis of fecal samples during in-home trials.

Conclusion

The current study demonstrated that in a population of healthy client-owned adult dogs, feeding extruded vegan diets containing only plant-based ingredients resulted in similar ATTD compared to a conventional animal-based diet with a similar nutrient profile. Digestibility studies with client-owned dogs can result in non-compliance with the study protocol, such as submitting a food diary. However, the current study found that the absence of food diaries did not affect the digestibility results, suggesting that this onerous requirement may not be a necessary component as long as clear feeding instructions are provided. Future research investigating vegan dog food should consider the effect of different nutrient profiles as well as length of feeding. Studies are also warranted to examine the effects of protocol compliance issues during in-home digestibility trials to develop a stronger methodology to improve reliability of results.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal studies were approved by the University of Guelph Animal Care Committee (AUP#4192) and the Research Ethics Board (Research Ethics Approval number 19-02-036), and were in accordance with institutional, provincial, and national guidelines for the care and use of animals and humans participating in research. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

BL: Writing – original draft. SD: Writing – review & editing. JA: Writing – review & editing. DG: Writing – review & editing. SB: Writing – review & editing. AV: Writing – review & editing.

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Conflict of interest

SD discloses they are the owner/operator of Dodds Veterinary Services, East Garafraxa, ON, Canada, providing veterinary nutritional consultation services to pet owners and within the pet food industry, and holds scientific advisory positions with pet food companies. AV discloses they are the Royal Canin Veterinary Diets Endowed Chair in Canine and Feline Clinical Nutrition at the University of Guelph Ontario Veterinary College, have financial support from companies within the pet food industry and hold scientific advisory positions with pet food companies. Author JA was employed by the company Petcurean Pet Nutrition Limited Partnership at the time of the study and is now employed by Archer-Daniels-Midland Co ADM.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim.2023.1288165/full#supplementary-material

References

AAFCO. (2019). *Association of American feed control officials: official publication* (Atlanta, GA, USA: Association of American Feed Control Officials).

Alvarenga, C. I., Aldrich, G. C., and Ou, Z. (2019). Comparison of four digestibility markers to estimate fecal output of dogs. *J.Animal. Sci.* 97, 1036–1041. doi: 10.1093/jas/skz020

AOAC (1990). *Official methods of analysis. 15th* (Arlington, VA: Assoc. Off. Anal. Chem.).

AOAC (1996). *Official methods of analysis. 15th* (Arlington, VA: Assoc. Off. Anal. Chem.).

AOAC (2005). *Official methods of analysis. 15th* (Arlington, VA: Assoc. Off. Anal. Chem.).

Axelsson, E., Ratnakumar, A., Arendt, M. L., Maqbool, K., Webster, M. T., Perloski, M., et al. (2013). The genomic signature of dog domestication reveals adaptation to a starch-rich diet. *Nature* 495 (7441), 360–364. doi: 10.1038/nature11837

Bednar, G., Murray, S., Patil, A., Flickinger, E., Merchen, N., and Fahey, G. (2000). Selected animal and plant protein sources affect nutrient digestibility and fecal characteristics of ileally cannulated dogs. *Arch. Anim. Nutr.* 53 (2), 127–140. doi: 10.1080/17450390009381942

Bednar, G. E., Patil, A. R., Murray, S. M., Grieshop, C. M., Merchen, N. R., and Fahey, G. C. (2001). Starch and fiber fractions in selected food and feed ingredients affect their small intestinal digestibility and fermentability and their large bowel fermentability in *vitro* in a canine mode. *J. Nutr.* 131 (2), 276–286. doi: 10.1093/jn/131.2.276

Borm, G. F., Fransen, J., and Lemmens, W. A. J. (2007). A simple sample size formula for analysis of covariance in randomized clinical trials. *J. Clin. Epidemiol.* 60 (12), 1234–1238. doi: 10.1016/j.jclinepi.2007.02.006

Buff, P. R., Carter, R. A., Bauer, J. E., and Kersey, J. H. (2014). Natural pet food: a review of natural diets and their impact on canine and feline physiology. *J. Anim. Science.* 92 (9), 3781–3379. doi: 10.2527/jas.2014-7789

Button, K. S., Ioannidis, J. P. A., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S. J., et al. (2013). Power failure: why small sample size undermines the reliability of neuroscience. *Nat. Rev. Neurosci.* 14 (5), 365–376. doi: 10.1038/nrn3475

Case, L. (2008). Perspectives on domestication: The history of our relationship with man's best friend. *J. Anim. Science.* 86 (11), 3245–3251. doi: 10.2527/jas.2008-1147

Clarys, P., Deliens, T., Huybrechts, I., Deriemaeker, P., Vanaelst, B., De Keyzer, W., et al. (2014). Comparison of nutritional quality of the vegan, vegetarian, semi-vegetarian, pesco-vegetarian and omnivorous diet. *Nutrients* 6 (3), 1318–1332. doi: 10.3390/nu6031318

Coelho, P. L., Kultima, J. R., Costea, P. I., Fournier, C., Pan, Y., Czarnecki-Maulden, G., et al. (2018). Similarity of the dog and human gut microbiomes in gene content and response to diet. *Microbiome* 6 (1), 72. doi: 10.1186/s40168-018-0450-3

Dichev, I. D., and Skinner, D. J. (2002)Large-sample evidence on the debt covenant hypothesis. Available at: http://www.jstor.org/stable/3542307 (Accessed March, 2023).

Dodd, S. A. S., Adolphe, J. L., Dewey, C., Khosa, D., Abood, S. K., and Verbrugghe, A. (2023). Efficacy of Vitamin D2 in maintaining serum total vitamin D concentrations and bone mineralization in adult dogs fed a plant-extruded diet in a three-month randomized trial. *Br. J. Nutr.* Submitted. doi: 10.1017/S0007114523001952

Dodd, S. A. S. (2022). A multimodal investigation into the suitability of plant-based diets for companion dogs and cats. [doctoral thesis] (Guelph, Ontario: The University of Guelph). Available at: https://atrium.lib.uoguelph.ca/xmlui/handle/10214/26672.

Dodd, S. A. S., Abood, S., Cave, N., Shoveller, A., Adolphe, J., and Verbrugghe, A. (2020). An observational study of pet feeding practices and how these have changed between 2008 and 2018. *Veterinary Rec.* 186 (19), 1–9. doi: 10.1136/vr.105828

Dodd, S. A. S., Adolphe, J. L., and Verbrugghe, A. (2018). Plant-based diets for dogs. *J. Am. Veterinary Med. Assoc.* 253 (11), 1425–1432. doi: 10.2460/javma.253.11.1425

Faber, J., and Fonseca, L. M. (2014). How sample size influences research outcomes. *Dental Press J. Orthodontics.* 19 (4), 27–29. doi: 10.1590/2176-9451.19.4.027-029.ebo

Fortes, C. M. L., Carciofi, S. ,. A. C., Sakomura, N. K., Kawauchi., I. M., and Vasconcellos, R. S. (2010). Digestibility and metabolizable energy of some carbohydrate sources for dogs. *Anim. Feed Sci. Technology.* 156 (3), 121–125. doi: 10.1016/j.anifeedsci.2010.01.009

Freel, T. A., McComb, A., and Koutsos, E. A. (2021). Digestibility and safety of dry black soldier fly larvae meal and black soldier fly larvae oil in dogs. *J. Anim. Science.* 99 (3), 1–8. doi: 10.1093/jas/skab047

Freeman, L., Becvarova, I., Cave, N., MacKay, C., Nguyen, P., Rama, B., et al. (2011). WSAVA nutritional assessment guidelines. *J. Feline Med. Surgery.* 13 (7), 516–525. doi: 10.1016/j.jfms.2011.05.0

German, A., Holden, S., Bissot, T., Hackett, R., and Biourge, V. (2007). Dietary energy restriction and successful weight loss in obese client-owned dogs. *J. Veterinary Internal Med.* 21 (6), 1174–1180. doi: 10.1892/06-280.1

Gilberto, D. B., Motzel, S. L., Bone, A. N., Burns, C. L., Zeoli, A. H., Lodge, K. E., et al. (2002). Use of three infusion pumps for postoperative administration of buprenorphine or morphine in dogs. *J. Am. Veterinary Med. Assoc.* 220 (11), 1655–1660. doi: 10.2460/javma.2002.220.1655

Golder, C., Weemhoff, J. L., and Jewell, D. E. (2020). Cats have increased protein digestibility as compared to dogs and improve their ability to absorb protein as dietary protein intake shifts from animal to plant sources. *Anim. (Basel).* 10 (3), 541–551. doi: 10.3390/ani10030541

Hagen-Plantinga, E. A., Bosch, G., and Hendriks, W. H. (2014). Practical approach to determine apparent digestibility of canine diets. *J. Nutr. Sci. (Cambridge).* 3, 1–4. doi: 10.1017/jns.2014.26

Hare, B., Wobber, V., and Wrangham, R. (2012). The self-domestication hypothesis: evolution of bonobo psychology is due to selection against aggression. *Anim. Behaviour.* 83 (3), 573–585. doi: 10.1016/j.anbehav.2011.12.007

Herstad, K. M., Gajardo, V. ,. K., Bakke, A. M., Moe, L., Ludvigsen, J., Rudi, K., et al. (2017). A diet change from dry food to beef induces reversible changes on the fecal microbiota in healthy, adult client-owned dogs. *BMC Veterinary Res.* 13 (1), 147–160. doi: 10.1186/s12917-017-1073-9

Kamboj, R., and Nanda, V. (2018). Proximate composition, nutritional profile and health benefits of legumes-A review. *Legume Research: Int. J.* 41 (3), 325–332. doi: 10.18805/LR-3748

Kanakubo, K., Fascetti, A., and Larsen, J. (2015). Assessment of protein and amino acid concentrations and labeling adequacy of commercial vegetarian diets formulated for dogs and cats. *J. Am. Veterinary Med. Assoc.* 247 (4), 385–392. doi: 10.2460/javma.247.4.385

Kendall, P., and Holme, D. (1982). Studies on the digestibility of soya bean products, cereals, cereal and plant by-products in diets of dogs. *J. Sci. Food Agriculture.* 33 (9), 813–822. doi: 10.1002/JSFA.2740330902

Knight, A. (2023). The relative benefits for environmental sustainability of vegan diets for dogs, cats, and people. *PloS One* 18 (10), 1–41. doi: 10.1371/journal.pone.0291791

Laflamme, D. (1997). Development and validation of a body condition score system for dogs. *Canine practice.* 22 (4), 10–15.

Laflamme, D. P. (2006). Understanding and managing obesity in dogs and cats. The Veterinary Clinics of North America. *Small Anim. Practice.* 36 (6), 1283–1295. doi: 10.1016/j.cvsm.2006.08.005

Laflamme, D. P., Abood, A. K., Fascetti, A. J., Fleeman, L. M., Freeman, L. M., Michel, K. E., et al. (2008). Pet feeding practices of dog and cat owners in the United States and Australia. *JAVMA* 232 (5), 687–694. doi: 10.2460/javma.232.5.687

Liversidge, B. D. (2023). The apparent total-tract nutrient digestibility, fecal microbiome, and fecal metabolome in adult health dogs fed an experimental plant-based diet compared to a conventional animal-based diet. [Master's thesis] (Ontario: The University of Guelph, Guelph).

Lyu, T., Liu, G., Zhang, H., Wang, L., Zhou, S., Dou, H., et al. (2018). Changes in feeding habits promoted the differentiation of the composition and function of gut microbiotas between domestic dogs (Canis lupus familiaris) and gray wolves (Canis lupus*). AMB Express.* 8 (1), 123–134. doi: 10.1186/s13568-018-0652-x

Moxham, G. (2001). Waltham feces scoring system - A tool for veterinarians and pet owners: how does your pet rate? *WALTHAM Focus* 11 (2), 24–25. Available at: https://nagonline.net/wp-content/uploads/2018/04/Waltham-Fecal-Scoring-Chart.pdf.

Murphy, K. F., German, A. J., Ruaux, C. G., Steiner, J. M., Williams, D. A., and Hall, E. J. (2003). Fecal α1-proteinase inhibitor concentration in dogs with chronic gastrointestinal disease. *Veterinary Clin. Pathology.* 32 (2), 67–72. doi: 10.1111/j.1939-165x.2003.tb00316.x

Nybroe, S., Astrup, A., and Bjørnvad, C. R. (2016). Dietary supplementation with flaxseed mucilage alone or in combination with calcium in dogs: effects on apparent digestibility of fat and energy and fecal characteristics. *Int. J. Obes.* 40 (12), 1884–1890. doi: 10.1038/ijo.2016.139

Osborne, D. R., and Voogt, P. (1978). *The analysis of nutrients in food* (London, UK: Academic Press Inc), 160–239.

Roberts, L. J., Oba, P. M., Utterback, P. L., Parsons, C. M., and Swanson, K. S. (2023). Amino acid digestibility and nitrogen-corrected true metabolizable energy of mildly cooked human-grade vegan dog foods using the precision-fed cecectomized and conventional rooster assays. *Trans. Anim. Sci.* 7 (1), 1-9. doi: 10.1093/tas/txad020

Röhrig, B., Du Prel, J. B., Wachtlin, D., Kwiecien, R., and Blettner, M. (2010). Sample size calculation in clinical trials: part 13 of a series on evaluation of scientific publications. *Deutsches Ärzteblatt Int.* 107 (31), 552–556. doi: 10.3238/arztebl.2010.0552

Santeramo, F. G., Carlucci, D., De Devitiis, B., Seccia, A., Stasi, A., Viscecchia, R., et al. (2018). Emerging trends in European food, diets, and food industry. *Food Res. Int.* 104, 39–47. doi: 10.1016/j.foodres.2017.10.039

Tanprasertsuk, J., Perry, L. M., Tate, D. E., Honaker, R. W., and Shmalberg, J. (2021). Apparent total tract nutrient digestibility and metabolizable energy estimation in commercial fresh and extruded dry kibble dog foods. *Trans. Anim. Science.* 5 (3), 1–9. doi: 10.1093/tas/txab071

Thompson, A. (2008). Ingredients: where pet food starts. *Topics Companion Anim. Med.* 23 (3), 127–132. doi: 10.1053/j.tcam.2008.04.004

Tran, Q. D., Hendriks, W. H., and van der Poel, A. F. (2008). Effects of extrusion processing on nutrients in dry pet food. *J. Sci. Food Agriculture.* 88 (9), 1487–1493. doi: 10.1002/jsfa.3247

Twomey, L. N., Pethick, D. W., Rowe, J. B., Choct, M., Plucke, J. R., Brown, W., et al. (2002). The use of sorghum and corn as alternatives to rice in dog foods. *J. Nutr.* 132 (6), 1704–1705. doi: 10.1093/jn/132.6.1704S

van der Kooij, M., Becvárová, I., Meyer, H., Teske, E., and Kooistra, H. (2014). Effects of an iodine-restricted food on client-owned cats with hyperthyroidism. *J. Feline Med. Surgery.* 16 (6), 491–498. doi: 10.1177/1098612X13512627

Wang, G., Zhai, W., Yang, H., Fan, R., Cao, X., Zhong, L., et al (2013). The genomics of selction in dogs and the parallel evolution between dogs and humans. *Nat. Commun.* 4, 1680. doi: 10.1038/ncomms2814

Weber, M. P., Stambouli, F., Martin, L. J., Dumon, H. J., Biourge, V. C., and Nguyen, P. G. (2002). Influence of age and body size on gastrointestinal transit time of radiopaque markers in healthy dogs. *Am. J. Veterinary Res.* 63 (5), 677–682. doi: 10.2460/ajvr.2002.63.677

WSAVA. (2013) *Body condition score (dog). Global Nutrition Toolkit*. Available at: https://wsava.org/wp-content/uploads/2020/01/Body-Condition-Score-Dog.pdf.

Yaissle, J., Holloway, C., and Buffington, C. A. (2004). Evaluation of owner education as a component of obesity treatment programs for dogs. *J. Am. Veterinary Med. Assoc.* 224 (12), 1932–1935. doi: 10.2460/javma.2004.224.1932

Yamka, R. M., Jamikorn, U., True, A. D., and Harmon, D. L. (2003). Evaluation of soyabean meal as a protein source in canine foods. *Anim. Feed Sci. Technology*. 109 (1), 121–132. doi: 10.1016/S0377-8401(03)00203-7

Zlatanova, D., Ahmed, A., Valasseva, A., and Genov, P. (2014). Adaptive diet strategy of the wolf (Canis Lupis L.) in Europe: a review. *Acta Zoological Bulgaria.* 66 (4), 439–452.