Apparent and true digestibility of macro and micro nutrients in adult maintenance dog foods containing either a majority of animal or vegetable proteins[1]

Cara L. Cargo-Froom[†], Ming Z. Fan[†], Guillaume Pfeuti[†], Christine Pendlebury [‡], Anna K. Shoveller[†,2]

[†]Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada; and [‡]Champion Pet Foods, Edmonton, AB, Canada

ABSTRACT: There is dearth of knowledge with regards to mineral digestibility of ingredients in canines, and current knowledge is focused on the digestibility of supplemented minerals, not on intrinsic mineral digestibility of ingredients. The objectives of the present study were to determine the apparent and true digestibility (TD) of macronutrients and micronutrients, and the total tract gastrointestinal endogenous nutrient outputs in canines fed either animal- or vegetable-based adult maintenance diets. Eight purpose bred Beagles (two intact males, six spayed females) of similar age (2.12 ± 0.35 yr, mean ± SD) and weight (9.92 ± 0.73 kg, mean ± SD) were pair housed in kennels but fed individually based on individual maintenance energy requirements. Two basal diets (animal and vegetable protein based) were formulated to meet nutritional requirements of adult canines. Two additional trial diets were created, using the basal diets, by diluting diets by 50% with anhydrous α-D-glucose to attempt to quantify endogenous mineral losses and enable calculation of TD. All diets contained titanium dioxide at 0.3% for calculations of nutrient digestibility. Dogs were provided with deionized water as their only source of water throughout the trial. Dogs in a specific kennel were randomly assigned to an experimental diet for 10 d (experimental period), and fecal samples were collected the last 4 d of each period. All dogs were fed all experimental diets in random order based on a 4 × 4 replicated Latin square design. Dogs fed intact diets had a higher apparent mineral digestibility compared to dogs fed diluted diets ($P < 0.05$). Apparent phosphorus digestibility was higher for dogs fed the diet 2 compared with the diet 1 ($P = 0.01$) and the diluted diets ($P < 0.001$). There was a trend towards a greater TD of Cu for dogs fed the diet 2 compared with the diet 1 ($P = 0.08$). P, Mg, Zn, and Mn true digestibilities were higher for dogs fed the diet 2 compared with the diet 1 ($P < 0.05$, $P = 0.01$, $P = 0.02$, $P = 0.009$, respectively). These results suggest that apparent and TD do not result in similar values. Further research should be conducted on TD in canines only if a better model is developed.

Key words: canine, digestibility, ingredients, minerals, protein

J. Anim. Sci. 2019.97:1010–1019
doi: 10.1093/jas/skz001

INTRODUCTION

The apparent digestibility of macronutrients from commonly used ingredients in dog food has been well documented in canines when compared with micronutrient digestibility, where there is a dearth of knowledge. Present research focuses on the digestibility of different supplemental mineral sources in the diet, yet data regarding the digestibility of intrinsic minerals of commonly used ingredients present in pet foods are minimal.

In growing pigs, mineral digestibilities between animal and vegetable ingredients differ. In general, ingredients from animal sources have greater

[1]This research was graciously funded by Champion Pet Foods and MITACs Accelerate.

[2]Corresponding author: ashovell@uoguelph.ca

Received August 4, 2018.
Accepted January 7, 2019.

digestibility of certain minerals (e.g., iron) but are often variable in micronutrient composition (Kim et al., 1995). Plants generally have a more stable composition of nutrients, but many vegetables and legumes contain anti-nutritional factors which bind minerals making them unavailable. However, heat processing can destroy anti-nutritional factors and improve the digestibility of these nutrients (Bohlke et al., 2005; Rehman and Shah, 2005). Additionally, as in the case of calcium, digestibility is inversely related with concentration in the diet in growing dogs (Hazewinkel et al., 1991). Moreover, apparent total tract digestibility underestimates true digestibility (TD) as endogenous mineral losses are not accounted for (e.g., calcium excreted from bone metabolism) (Weremko et al., 1997). True mineral digestibility of a diet has been calculated using an indigestible marker and the same diet with diluted nutrient content (Fang et al., 2007).

The objectives of this study were to compare the digestibility of intrinsic sources of minerals in adult dogs. We hypothesized that the diet 1 will have higher mineral digestibility compared to the diet 2 and that the diluted diets will have increased digestibility compared with the intact.

MATERIALS AND METHODS

Animals and Housing

All procedures were approved by the Animal Care Committee at the University of Guelph, ON (AUP #3543). Eight purpose bred Beagles (two intact males, six spayed females) of similar age (2.12 ± 0.35 yr, mean ± SD) and weight (9.92 ± 0.73 kg, mean ± SD) were used. Dogs were provided by Marshall Farms (North Rose, NY) to the Ontario Veterinary College and housed at the Central Animal Facility (University of Guelph, Guelph, ON, Canada). Dogs were pair housed in kennels (121.9 cm × 190.5 cm) that had the ability to be sectioned in half by a sliding door. Kennels were constructed of stainless steel walls and tenderfoot flooring (Tenderfoot, Minneapolis, MN). All kennels were located in the same environmentally controlled room (20 ± 1.5 °C, 40 ± 10% relative humidity), with a 12 h light:12 h dark cycle. Kennels were rinsed daily between 0900 and 1200 hours and were disinfected every 10 d with Peroxiguard (Bayer, Mississauga, ON, Canada). Dogs were provided enrichment and walked in pairs between 0900 and 1200 hours for 20 min five times per week (weekdays) and 10 min two times per week (weekends). Additional socialization was provided by the primary researcher between 0900 and 1200 hours in the form of walks, individual interaction, and paired interactions within kennel groups.

Experimental Diet and Designs

Four treatment diets were allocated for this study; two intact diets: animal protein ingredient-based diet (**diet 1**), vegetable protein ingredient-based diet (**diet 2**), and two diluted diets: animal or vegetable protein ingredient-based diet at a 50% dilution on a weight basis with anhydrous α-D-glucose (Sigma-Aldrich, St. Louis, MO) (**diet 4** and **diet 3**, respectively). Both diets included animal and vegetable ingredients with a focus on animal protein vs. vegetable protein to differentiate the treatments. Intact diets were formulated and made by Champion Pet Foods (Edmonton, AB, Canada). Raw ingredients were weighed out, mixed, and extruded to create kibble (Morinville, AB, Canada). Intact diets were formulated to have similar nutrient profiles (Table 1) to enable evaluation of different ingredients. The intact diets were formulated to meet or exceed the Association of American Feed Control Officials (**AAFCO**) nutrient recommendations (AAFCO, 2014), but they were not formulated for commercial use. Nutrient composition of diets was similar within intact and diluted diets, with the exception of calcium and phosphorus (Table 2). Concentrations of calcium (**Ca**) and phosphorus (**P**) were not equalized with supplemental minerals because the goal of the study was to evaluate the digestibility of intrinsic minerals.

Diets and kennels were randomized with each dog cycling through the four diets based on a 4 × 4 replicated Latin square design with 4 kennels and 4 periods, with 2 dogs per kennel. Each period consisted of 10 d, with 6 d of acclimation to the diet immediately followed by 4 d of fecal sample collections. Dogs were fasted overnight and weighed on days 0 and 6. The amount of diet provided was based on energy density of each diet and maintenance energy requirements for each dog. Maintenance energy requirements were calculated based on current BW, historical BWs, and historical feeding amounts (27.60 ± 8.59 kcal ME/kg BW). Dogs were fed at 95% maintenance energy to ensure consumption of all food provided. Treatment diets were weighed out for each dog by individual meal, and titanium dioxide (**TiO_2**; Sigma-Aldrich) was added as an indigestible marker at an inclusion rate of 0.3% of each meal. Treatment diets with TiO_2 were weighed and prepared in advanced for each 10 d phase. Warm deionized water was added to the fully prepared

Table 1. Ingredient composition of intact experimental diets fed to 8 healthy adult dogs[1]

Ingredients, g/kg diet as fed	Animal based	Vegetable based
Fresh beef (liver and trim)	150.0	—
Fresh potato	130.0	130.0
Corn gluten meal	—	130.0
Chickpeas and lentils	121.6	120.6
Fresh chicken	120.0	—
Green and yellow peas	85.0	190.0
Fresh fish blend	85.0	—
Soybean meal	—	90.0
Chicken meal	75.0	90.0
Low ash herring meal	50.0	—
Sweet potato	50.0	50.0
C15066 Chicken Liquid Palatant	25.0	25.0
Chicken Fat Category 3 Spray	25.0	11.5
Fresh whole egg	20.0	—
Chicken dry palatant	5.0	5.0
Fresh veggie and fruit blend	5.0	5.0
Enticer B28009	5.0	5.0
Egg powder	4.0	-
Kelp – Tasco	1.5	1.5
Salt	1.0	1.0
CPF vitamin ADE	1.0	1.0
Natural antioxidant liquid	0.5	0.5
Natural antioxidant dry	0.3	0.3
Acana dog botanical blend	0.1	0.1
Bacteria blend	0.03	0.03

[1]Intact ingredient diets were diluted by 50% on a weight basis with α-anhydrous-D-glucose to create dilute kibble diets.

dietary treatments (including TiO_2) immediately before feeding. Addition of water allowed added glucose in diluted diets to dissolve reducing potential glucose losses. Water was added to intact diets to ensure consistent preparation among all treatments. The addition of water also allowed for the diets to be mixed using a hand blender. All diets were mixed for a standardized amount of time (2 min) to form a homogenous mixture and to reduce the ability of the dogs to selectively eat portions of the diets. All mixing equipment was rinsed with deionized water to ensure no treatment or TiO_2 was lost. Dogs were fed individually at 0800 and 1300 hours daily, and no food refusals occurred. Feeding bowls were cleaned with hot water and commercial dishwashing liquid between meals. Dogs had *ad libitum* access to fresh deionized water at all times. Deionized water was used to ensure dogs did not have an unregulated secondary source of minerals.

Sample Collections and Analysis

Treatment diets were sampled in triplicate during the trial. Sample collections of each fully prepared diet were taken by sampling blended diets containing TiO_2 and water. Samples were stored and frozen in sample collection bags at −20 °C until analysis.

During sampling days, fecal samples were collected starting at 0800 hours on day 6. Dogs remained housed in the same kennels but were divided individually within the kennel from 0000 until 0900 hours to allow for individual fecal collections. Feces were also collected throughout the day during walks and eliminations while researcher provided enrichment for each individual dog. However, these research dogs preferred to eliminate first thing in the morning and did not eliminate regularly during walks and enrichments, therefore many of the feces collected were during individual housing hours. Feces were frozen in sample bags for each dog at −20 °C until analysis. Feces were pooled by period for each individual dog. After freeze drying, diet and fecal samples were ground using a Wiley mill (1 mm screen, Thomas Scientific, Swedesboror, NJ). DM was analyzed by drying samples in an oven at 105 °C (Fisher Scientific Isotemp Oven, Markham, ON, Canada) overnight, ash content was calculated by combustion of samples at 550 °C (Fisher Scientific Isotemp Muffle Furnace, Markham, ON, Canada) for 10 h. Concentration of TiO_2 in feces (duplicate) and diets (quadruplicate) was determined according to Myers et al. (2004) with minimal adaptation (sample digestion for 24 h at 120 °C in 10 mL tubes and addition of H_2O_2 to supernatant in micro well plate). Absorbance values were read using a BioTek Powerwave XS spectrophotometer (BioTek, Winooski, VT) at 408 nm. Crude protein (N × 6.25) content was determined by nitrogen analysis using macro-Kjeldahl method (AOAC, 1995) using a Kjeltec protein analyzer (Model #8200, Tecator, Hoganas, Sweden). Crude fat analysis was performed using petroleum ether with an Ankom XT20 fat analyser (Ankom Technology, Macedon, NY).

AA content of diet and fecal samples were analyzed using ultra performance liquid chromatography (**UPLC**) (Waters Corporation, Milford, MA). Approximately 0.05 g of dried sample was hydrolyzed in a test tube with 450 µL 6 N hydrochloric acid (**HCl**) and 1% phenol for 24 h at 110 °C under prepurified nitrogen atmosphere. AA standards and hydrolyzed diets and fecal samples (10 µL) were derivatized by ACCQTag Ultra derivatization kit (Waters Corporation) according to Boogers et al. (2008). The derivatized AA were separated using UPLC with UV detection (260 nm) with a cycle time of 10 min per sample. Derivatized AA

Table 2. Analyzed nutrient of composition of treatment diets fed to eight healthy adult dogs

Analyzed nutrient, as fed	Diet 1	Diet 2	Diet 3	Diet 4
Metabolizable energy, kcal/kg[1]	3,231	3,394	3,340	3,489
DM, %	96.17	96.75	93.60	92.89
OM, %	89.94	91.61	91.07	89.53
CP, %	33.01	32.64	15.16	15.54
Crude fat, %	11.45	13.32	6.50	4.57
Crude fiber[2], %	3.4	2.8	1.7[3]	1.4[3]
Total dietary fiber, %	10.9	10.4	5.4[4]	5.2[4]
Calcium, %	0.89	0.53	0.27	0.50
Phosphorus, %	0.84	0.65	0.35	0.46
Calcium: phosphorus	1	0.8	0.7	1
Potassium, %	1.06	1.04	0.48	0.53
Sodium, %	0.27	0.14	0.12	0.14
Magnesium, %	0.12	0.12	0.06	0.06
Copper, mg/kg	14.60	11.83	5.91	7.30
Zinc, mg/kg	72.63	53.00	34.13	35.27
Iron, mg/kg	129.03	121.65	63.53	66.83
Manganese, mg/kg	13.70	15.13	7.57	6.85
AAs				
Alanine, %CP	1.57	1.81	0.81	0.87
Arginine, %CP	1.98	1.98	0.76	0.90
Asparagine, %CP	2.56	2.65	1.17	1.43
Glycine, %CP	1.98	1.98	0.76	0.90
Histidine, %CP	2.56	2.65	1.17	1.43
Isoleucine, %CP	1.23	1.34	0.58	0.64
Leucine, %CP	2.16	3.13	1.36	1.13
Lysine, %CP	1.58	1.35	0.57	0.88
Phenylalanine, %CP	1.46	1.92	0.76	0.68
Proline, %CP	1.50	1.98	0.87	0.82
Serine, %CP	1.30	1.58	0.66	0.65
Threonine, %CP	1.14	1.20	0.49	0.57
Tyrosine, %CP	1.02	1.34	0.45	0.41
Valine, %CP	1.53	1.60	0.69	0.80

[1]Metabolizable energy calculated using the modified Atwater equation.

[2]Crude fiber determined through Near Infrared Spectroscopy by Champion Petfoods.

[3]Estimated concentration based off of a 50% nutrient dilution (required for energy calculation).

[4]Estimated concentration based off of a 50% nutrient dilution.

(1 µL injection volume) were separated in a column (2.1 × 2000 mm, 1.7 µL) maintained at 55 °C. AA peak areas were compared with known standards and analyzed using Waters Empower 2 Software (Waters Corporation).

Minerals were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES). Sample preparation was adapted based on procedures outlined by Topper and Kotuby-Amacher (1990) and the US EPA (2015). Approximately 0.5 g of sample was weighed in an acid washed Teflon digestion vessel, along with a blank and a standard reference material. In a fume hood, 9 mL of 70% nitric acid (trace metal grade) and 3 mL of HCl (trace metal grade) were added to the samples and swirled every 30 min until bubbles subsided. Samples sat for a total of 7 h during digestion and subsequently placed in a cool oven. Oven temperature was raised to 120 °C, and samples were digested overnight. Samples were filtered (Whatman 42 filter paper) into 50-mL acid washed flasks, and brought up to volume with deionized water (18.3 MΩ/cm). Digested samples were analyzed by ICP-OES using a Varian VISTA Pro (Varian, Palo Alto, CA) in the Environmental Sciences Department (University of Guelph) using axially viewed plasma.

Digestibility Calculations

Apparent digestibility (**AD**, %) was calculated using the marker technique as follows:

$$AD = 100 - \left(\frac{\left[\frac{N_F}{M_D}\right]}{\left[\frac{N_D}{M_F}\right]} \times 100 \right) \quad (1)$$

where N_D is the concentration of nutrient in the diet (%), N_F is the concentration of nutrient in the feces (%), M_D is the concentration of marker (TiO_2) in the diet (%), and M_F is the concentration of marker (TiO_2) in the feces (%). All values were on a DM basis.

Based on the AD of nutrients, TD (%) for minerals was calculated according to eq. 2 used by Fang et al. (2007) with minor modifications:

$$TD = 100 \times \left(\frac{[N_H - F_H] - [N_L - F_L]}{[N_H - N_L]} \right) \quad (2)$$

where N_H is the amount of nutrient in the intact diet (g/kg DM), F_H is the amount of nutrient in the fecal output of the intact diet (g/kg DM), N_L is the average amount of nutrient in the diluted diet (g/kg DM), F_L is the average amount of nutrient in the fecal output of the diluted diet (g/kg DM).

Endogenous losses ($\mathbf{E_L}$, g/kg DMI) were calcualted using eq. 3 by Fan et al. (2008):

$$E_L = 100 \times ([\text{TD-AD}] \times N_{DI}) \quad (3)$$

where TD is the true digestibility of nutrient (%), AD is the apparent digestibility of nutrient (%), N_{DI} is the nutrient content of the diet (g/kg DM).

Statistical Analysis

BW data were analyzed using a mixed model in SAS (SAS Inst. Inc., Cary, NC) where diet, day, and period were fixed effects, and dog was a random effect. Diet × period and diet × day interactions were assessed, deemed insignificant, and removed from the model. Apparent and TD, and endogenous losses data were analyzed using a PROC MIXED procedure of SAS (version 9.4, SAS Inst. Inc.) where diet and period were included as fixed effects and dog as a random effect. Individual means were compared using least square means and pdiff. Significance was declared at $P < 0.05$, with trends are discussed at $0.10 > P > 0.05$. Data points which were determined to be outliers, through the proc univariate command in SAS (SAS Inst. Inc.), were omitted from the dataset and analysis. Data are presented as means and standard error means (SEM).

RESULTS

All dogs remained healthy throughout the experiment. BW for all dogs was not different between diets ($P = 0.90$) or periods ($P = 0.40$). Interaction diet × period, and diet × day were not significant ($P > 0.10$).

Apparent Digestibility

AD values are presented in Table 3. DM and OM digestibility were higher for diet 2 compared with all other diets ($P = 0.02$). CP digestibility was greater in the intact diets (diets 1 and 2) when compared with the diluted diets (diets 3 and 4) ($P < 0.0001$). Crude fat digestibility was greater in the intact diets when compared with the diluted diets ($P < 0.0001$). Diet 3 had a greater crude fat digestibility than diet 4 ($P = 0.01$).

Apparent digestibilities for all minerals were greater in the intact diets compared with the diluted diets ($P < 0.001$), excluding sodium (**Na**) ($P = 0.06$). With the exception of P, all mineral digestibilities were not different between diets 1 and 2 or diets 3 and 4. Phosphorus digestibility was greater for diet 2 compared with diet 1 ($P = 0.01$). With the exception of alanine, all AA digestibilities were greater in the intact diets compared with the diluted diets ($P < 0.0002$). Alanine and leucine digestibility of diet 2 was greater compared with all other diets ($P = 0.0003$).

True Digestibility

TD values for the intact kibble diets are presented in Table 4. DM TD was greater for diet 2 compared with diet 1 ($P = 0.03$). There was no difference between CP TD for diets 1 and 2 ($P = 0.11$). There was no difference between the true digestibilities of Ca, potassium (**K**), Na, and iron (**Fe**) ($P \geq 0.10$). There was a trend toward a greater TD of copper (**Cu**) for diet 2 compared with diet 1 ($P = 0.08$). True digestibilities for P, magnesium (**Mg**), zinc (**Zn**), and manganese (**Mn**) were greater for diet 2 compared with diet 1 ($P < 0.05$).

Endogenous Losses

Endogenous loss values, calculated by eq. 3, are presented in Table 5. Endogenous losses were greater in diets 1 and 4 compared with diets 2 and 3 ($P < 0.05$) for Na, Mg, Zn, and Mn. Endogenous Ca loss between the diets 4 and 2 was significant ($P = 0.046$).

Table 3. Effects of experimental diets on apparent total tract digestibility of different nutrients

Nutrient, %	Diet 1	Diet 2	Diet 3	Diet 4	SEM	*P*–value
DM	86.94[a]	90.69[b]	86.65[a]	84.65[a]	0.7	0.02
OM	89.22[a]	92.28[b]	89.51[a]	87.72[a]	0.5	0.02
CP	88.33[a]	92.28[a]	75.86[b]	73.70[b]	1.9	<0.001
Crude fat	97.13[a]	98.02[a]	92.18[b]	89.47[c]	0.9	<0.001
Calcium	42.16[a]	50.07[a]	−22.73[b]	−1.68[b]	8.0	<0.001
Phosphorus	61.27[a]	78.18[b]	36.81[c]	36.91[c]	4.6	<0.0001
Potassium	96.39[a]	97.15[a]	92.03[b]	92.08[b]	0.7	0.003
Sodium	87.07[a]	84.85[ab]	76.45[b]	83.21[b]	2.4	0.06
Magnesium	47.51[a]	64.01[a]	−14.69[b]	−31.32[b]	8.1	0.0005
Copper	47.47[a]	54.00[a]	−5.64[b]	5.26[b]	7.4	0.002
Zinc	47.42[a]	54.99[a]	18.77[b]	2.81[b]	5.4	0.001
Iron	44.90[a]	52.95[a]	4.61[b]	5.21[b]	6.2	0.003
Manganese	33.13[a]	49.12[a]	−21.03[b]	−31.32[b]	9.1	0.0006
AAs						
Alanine	81.49[a]	92.69[b]	78.54[a]	78.73[a]	1.5	<0.0001
Arginine	93.78[a]	95.40[a]	84.14[b]	84.80[b]	1.2	<0.0001
Asparagine	88.60[a]	92.16[a]	76.88[b]	77.69[b]	1.6	<0.0001
Glycine	90.87[a]	92.31[a]	77.32[b]	81.75[b]	1.5	<0.0001
Histidine	91.33[a]	94.09[a]	80.35[b]	80.15[b]	1.5	<0.0001
Isoleucine	90.99[a]	93.13[a]	82.13[b]	80.17[b]	1.4	<0.0001
Leucine	90.82[a]	94.50[b]	83.05[c]	80.40[c]	1.4	0.0003
Lysine	89.62[a]	91.17[b]	86.71[a]	86.50[a]	0.7	<0.0001
Phenylalanine	90.92[a]	94.56[a]	81.50[b]	79.22[b]	1.5	<0.0001
Proline	91.42[a]	93.97[a]	82.20[b]	81.77[b]	1.3	<0.0001
Serine	88.55[a]	92.78[a]	76.74[b]	74.09[b]	1.9	0.0002
Threonine	88.90[a]	91.51[a]	72.87[b]	74.55[b]	2	<0.0001
Tyrosine	88.81[a]	93.38[a]	72.28[b]	68.91[b]	2.4	<0.0001
Valine	90.78[a]	92.58[a]	78.17[b]	81.68[b]	1.5	0.03

[a–c]Means within rows with no common superscript differ ($P < 0.05$).

Table 4. Effects of experimental diets on true total tract digestibility of different nutrients

Nutrient, %	Diet 1 $n = 6$	Diet 2 $n = 6$	SEM[1]	*P*-value
DM	148.61[a]	206.19[b]	16.4	0.03
CP	101.57[a]	106.02[a]	1.2	0.11
Calcium	87.35[a]	112.61[a]	7.4	0.12
Phosphorus	118.61[a]	178.18[b]	9.3	0.001
Potassium	100.94[a]	101.77[a]	0.6	0.59
Sodium	98.43[a]	132.13[a]	13.6	0.33
Magnesium	86.14[a]	138.42[b]	9.1	0.01
Copper	88.71[a]	111.45[a]	7.6	0.08
Zinc	105.96[a]	159.22[b]	10.8	0.02
Iron	115.26[a]	140.10[a]	8.0	0.12
Manganese	97.58[a]	171.44[b]	13.2	0.009

[a,b]Means within rows with no common superscript differ ($P < 0.05$).

[1]SEM based on six observations.

DISCUSSION

To the authors' knowledge, this study is the first to report AD and TD of minerals supplied in the diet exclusively through ingredient sources and fed to dogs. The intact diets for this study were formulated to have a similar nutrient composition, with the exception of P and Ca which were lower in the vegetable ingredient-based diet. While Ca and P concentrations could have been greater in the vegetable-based diet through supplementation, this would have defeated the purpose of the study. For

Table 5. Endogenous mineral losses associated with experimental diets

Nutrient, g/kg DM	Diet 1	Diet 4	Diet 2	Diet 3	SEM[1]	*P*-value
Calcium	4.17[ab]	4.81[a]	3.43[b]	3.90[ab]	0.22	0.19
Phosphorus	2.85[a]	2.85[a]	3.56[a]	3.56[a]	0.16	0.09
Potassium	0.50[a]	0.50[a]	0.50[a]	0.50[a]	0.03	0.92
Sodium	0.13[a]	0.13[a]	0.71[b]	0.74[b]	0.08	<0.001
Magnesium	0.49[a]	0.49[a]	0.94[b]	0.94[b]	0.06	0.004
Copper	0.006[a]	0.006[a]	0.007[a]	0.007[a]	0.0004	0.96
Zinc	0.04[ac]	0.04[a]	0.06[b]	0.051[bc]	0.002	0.01
Iron	0.08[a]	0.07[a]	0.08[a]	0.08[a]	0.003	0.63
Manganese	0.009[a]	0.009[a]	0.02[b]	0.01[c]	0.001	<0.001

[a–c]Means within the same rows with no common superscript differ ($P < 0.05$).

[1]Pooled SEM based on six observations.

this reason, differences between diets 1 and 2 with regards to Ca and P digestibility were considered when interpreting the results.

The higher apparent digestibility of P in diet 2 compared to diet 1 may have four explanations. First, during processing (e.g., extrusion) anti-nutritional factors are destroyed, allowing bound nutrients to become available. Extrusion temperatures between 120 – 140 ˚C are effective at increasing the nutritional value of legumes, through the destruction of anti-nutritional factors, particularly phytic acid (phytate), without greatly affecting other major nutrients (e.g., protein, Ca; Alonso et al., 1998; Purushotham et al., 2007). The second explanation could be related to the lower P concentration in the vegetable based ingredient diet compared to the animal based ingredient diet. There is an inverse relationship with Ca concentration of a diet and Ca digestibility/absorption in growing and adult dogs (Hazewinkel et al., 1991; Kastenmayer et al., 2002). If other minerals follow a similar digestibility relationship to Ca, this may support the higher P digestibility associated with the diet 2. Third, Stein et al. (2011) reported decreased P absorption when Ca in the diet increased and concluded that apparent digestibility of P is greatest when Ca:P ratio in the diet is at 1.1:1 ratio or slightly lower. Diets 2 and 3 had a lower Ca:P ratio compared to diets diets 1 and 4. Cumulatively, these studies suggest that focusing on the Ca:P ratio in commercial dog food is a critical piece to improving mineral digestibility of these two nutrients. The current results also suggest that current supplementation practices may be limiting the efficiency of digestibility of these macro minerals. Further research should focus on the ideal amount for common mineral supplements in combination with common dog food ingredients to maximize digestibility of minerals and limit excess. Finally, the fourth explanation could be related to the total dietary fiber content of the diet and fermentable material. Soybeans, and other similar legumes, contain 5% to 15% oligosaccharide content, including highly fermentable galacto-oligosaccharides (e.g., raffinose; Chilomer et al., 2011; Fan et al., 2015; Hall et al., 2017). It is probable based on leguminous ingredients that the treatment diets 1 and 2 contain 1% to 3% and 2% to 6% of these highly fermentable materials, respectively. Based on ingredient inclusion, the amount of fermentable material in the treatment diets are greater compared with prebiotic amounts included in companion animal diets, potentially impacting mineral digestibility. However, a study in dogs by Beynen et al. (2002) determined that supplemented oligofructose, a nondigestible oligosaccharide (**NDO**), increased Ca and Mg absorption, but did not alter P absorption. Total dietary fiber and NDO affect gut health and macro nutrient digestion in dogs; however, there is very little data to support the effects of total dietary fiber and NDO on mineral digestibility in dogs (Swanson et al., 2002a, b; Propst et al., 2003). The effects of total dietary fiber of common pet food ingredients on gastrointestinal health (i.e., fermentation and bacterial populations) and the relationship with mineral uptake/solubility is an obvious next step in understanding these complex interactions.

Hazewinkel et al. (1991) fed growing Great Danes foods which varied in Ca and P content. Control dogs fed normal Ca levels (1.1%) absorbed 45% to 66% of calcium provided. Ca levels fed at higher (3.3 %) and lower (0.55 %) amounts changed Ca absorption to 23% to 43% and 70% to 97%, respectively, based on apparent digestibility. We expected to see this change in digestibility reflected in our results when comparing intact vs. diluted diets, assuming that the diluted diets would have a higher apparent digestibility due to the decrease in nutrient concentration. However, this was not the case, the apparent digestibility for the diluted

diets was significantly lower compared with the intact diets. A study by Schmitt et al. (2018) fed adult dogs a low Ca diet supplied with calcium carbonate to meet the minimal requirements of the animals for 28 wk to assess if long-term adaptation to low calcium intake occurs. Similar to the present results, Schmitt et al. (2018) found negative apparent Ca digestibility occurred in dogs fed the low calcium diet, with fecal excretion of Ca consistently greater than Ca intake. Additionally, serum Ca levels were not affected when dogs were fed the low Ca diet; to maintain serum Ca levels without increased Ca absorption, bone resorption of Ca would need to occur (Schmitt et al., 2018). The results by Schmitt et al. (2018) suggest that dogs do not adapt intestinal absorption of Ca, and instead rely on other methods of calcium homeostasis when challenged with a low Ca diet. These results are supported by our endogenous loss calculations, with the exception of manganese, there was no significant difference between diets 1 and 4, and diets 2 and 3 in regards to mineral endogenous losses. If the dogs were indeed adapting to the reduced nutrient concentration in the diluted diets, we would have expected to see significantly lower values for endogenous losses. Since it appears that dogs do not adapt to the reduced nutrient concentration, even in the case of a longer study/adaptation period, TD studies may be hard to conduct in canines using a reduced nutrient content method.

In growing pigs, apparent digestibility of nutrients is often underestimated compared with TD (Weremko et al., 1997). While the TD values found in the present study are higher compared with the apparent digestibility values, many of the values calculated were exceedingly high. Though these values may appear extreme, they may in fact be correct on a calculation basis only. Based on evidence by Schmitt et al. (2018), if the same occurs for other minerals (i.e., no increase in absorption) as it does with Ca, then a low mineral diet would yield reduced or negative apparent digestibility values. This in turn would account for the extremely high TD values calculated. Unlike the pigs in the study by Fang et al. (2007) who adapted to the diluted diets, it appears the dogs did not adapt to their reduced mineral intake, resulting in true digestibility values which are most likely inaccurate, suggesting that the substitution method does not work in canine studies.

Our apparent digestibility values for macro nutrients are similar to those reported in literature. Clapper et al. (2001) assessed the effects of soybean fractions incorporated into extruded kibble on total tract digestibility fed to dogs. Mean apparent DM, OM, CP, and crude fat digestibility ranged from 79% to 82%, 81% to 87%, 76% to 87%, and 92% to 96%. In canned canine diets, texturized vegetable protein inclusion at different rates had apparent digestibility ranges of 83% to 87%, 80% to 86%, and 98% for DM, CP, and crude fat, respectively (Hill et al., 2001). The significantly higher DM and OM digestibility of diet 2 is most likely due to the inverse relationship between DM/OM digestibility and crude fiber content of diets, as crude fiber content increases DM/OM digestibility decreases (Burrows et al., 1982; Castrillo et al., 2001). Crude fat digestibility was significantly different between diets 3 and 4 and may be linked to crude fiber and/or the addition of glucose to the diluted diets. Diets with additional fiber had lower crude fat digestibility (Burkhalter et al., 2001). However, rather than directly altering the solubility of lipids and their absorption, fiber affects the physical properties (e.g., viscosity) of intestinal contents (Furda et al., 1990). These physical changes affect the rate of absorption; a decrease in gastro-intestinal transit time (**GITT**) is associated with a decrease in lipid absorption (Furda et al., 1990). Altered GITT in the study could be attributed to the large increase of glucose in the diluted diets. However, for glucose to have a direct effect on GITT there would need to be impaired glucose transport, allowing glucose to remain in the ileum and large intestine leading to osmotic diarrhea and rapid fermentation of the sugars by microbes (Southgate, 1989). While none of the dogs experienced diarrhea, a decrease in GITT may still have occurred, reducing digesta exposure to the luminal wall and impairing absorption.

These results confirm that both animal- and vegetable-based ingredient diets can supply the required minerals for adult dogs. AD of minerals for both animal- and vegetable-based ingredient diets is similar to values reported in literature. This could suggest that a reduction or elimination of mineral premixes in adult maintenance dog food is possible. AD results also demonstrate that a vegetable ingredient-based diet can match or exceed the digestibility of nutrients when compared with an animal-based ingredient diet. While the substitution method has been used to determine TD of P in growing pigs, it appears that this method may not be suitable to study the TD of minerals in dogs. Further research is required to properly assess TD of intrinsic minerals from common pet food ingredients in adult dogs if a proper method can be developed and used, and if so, whether TD can be used as a valuable methodology in adult canines.

Conflict of interest statement. None declared.

ACKNOWLEDGMENTS

The authors thank the staff at the Central Animal Facility (University of Guelph) and the Clinical Nutrition Department (Ontario Veterinary College, University of Guelph) for facilitating the research and resource sharing, and also to Brad Jacobs for providing his expertise as a veterinary technician.

LITERATURE CITED

Alonso, R., E. Orúe, and F. Marzo. 1998. Effects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. Food Chem. 63:505–512. doi:10.1016/S0308-8146(98)00037-5

AOAC. 1995. Official methods of analysis. 16th ed. Arlington, VA: AOAC International.

Association of American Feed Control Officials (AAFCO). 2014. Proposed revisions for official publication: dog and cat nutrient profiles. West Lafayette, IN: Association of American Feed Control Officials.

Beynen, A. C., J. C. Baas, P. E. Hoekemeijer, H. J. Kappert, M. H. Bakker, J. P. Koopman, and A. G. Lemmens. 2002. Faecal bacterial profile, nitrogen excretion and mineral absorption in healthy dogs fed supplemental oligofructose. J. Anim. Physiol. Anim. Nutr. (Berl.) 86:298–305. doi:10.1046/j.1439-0396.2002.00386.x

Bohlke, R. A., R. C. Thaler, and H. H. Stein. 2005. Calcium, phosphorus, and amino acid digestibility in low-phytate corn, normal corn, and soybean meal by growing pigs. J. Anim. Sci. 83:2396–2403. doi:10.2527/2005.83102396x

Boogers, I., W. Plugge, Y. Q. Stokkermans, and A. L. Duchateau. 2008. Ultra-performance liquid chromatographic analysis of amino acids in protein hydrolysates using an automated pre-column derivatisation method. J. Chromatogr. A 1189:406–409. doi:10.1016/j.chroma.2007.11.052

Burkhalter, T. M., N. R. Merchen, L. L. Bauer, S. M. Murray, A. R. Patil, J. L. Brent, Jr, and G. C. Fahey, Jr. 2001. The ratio of insoluble to soluble fiber components in soybean hulls affects ileal and total-tract nutrient digestibilities and fecal characteristics of dogs. J. Nutr. 131:1978–1985. doi:10.1093/jn/131.7.1978

Burrows, C. F., D. S. Kronfeld, C. A. Banta, and A. M. Merritt. 1982. Effects of fiber on digestibility and transit time in dogs. J. Nutr. 112:1726–1732. doi:10.1093/jn/112.9.1726

Castrillo, C., F. Vicente, and J. A. Guada. 2001. The effect of crude fibre on apparent digestibility and digestible energy content of extruded dog foods. J. Anim. Physiol. Anim. Nutr. (Berl.) 85:231–236. doi:10.1046/j.1439-0396.2001.00329.x

Chilomer, K., K. Zaleska, D. Ciesiołka, P. Gulewicz, A. Frankiewicz, and K. Gulewicz. 2011. Changes in the alkaloid, α-galactoside and protein fractions content during germination of different lupin species. Acta Soc. Bot. Pol.. 79:11–20. doi:10.5586/asbp.2010.002

Clapper, G. M., C. M. Grieshop, N. R. Merchen, J. C. Russett, J. L. Brent, Jr, and G. C. Fahey, Jr. 2001. Ileal and total tract nutrient digestibilities and fecal characteristics of dogs as affected by soybean protein inclusion in dry, extruded diets. J. Anim. Sci. 79:1523–1532. doi:10.2527/2001.7961523x

Fan, M., Y. Shen, Y. L. Yin, Z. R. Wang, Z. Y. Wang, T. Li, T. Rideout, R. Huang, T. Archbold, C. Yang, et al. 2008. Methodological considerations for measuring phosphorus utilization in pigs. Math. Model. Anim. Nutr. 370–397. doi:10.1079/9781845933548.0370

Fan, P. H., M. T. Zang, and J. Xing. 2015. Oligosaccharides composition in eight food legumes species as detected by high-resolution mass spectrometry. J. Sci. Food Agric. 95:2228–2236. doi:10.1002/jsfa.6940

Fang, R. J., Y. L. Yin, K. N. Wang, J. H. He, Q. H. Chen, T. J. Li, M. Z. Fan, and G. Wu. 2007. Comparison of the regression analysis technique and the substitution method for the determination of true phosphorus digestibility and faecal endogenous phosphorus losses associated with feed ingredients for growing pigs. Livest. Sci. 109:251–254. doi:10.1016/j.livsci.2007.01.108

Furda, I., C. J. Brine, and American Chemical Society, eds. 1990. New developments in dietary fiber: physiological, physicochemical, and analytical aspects. New York: Plenum Press.

Hall, C., C. Hillen, and J. G. Robinson. 2017. Composition, nutritional value, and health benefits of pulses. Cereal Chem. 94:11–31. doi:10.1094/CCHEM-03-16-0069-FI

Hazewinkel, H. A., W. E. Van den Brom, A. T. Van 'T Klooster, G. Voorhout, and A. Van Wees. 1991. Calcium metabolism in great dane dogs fed diets with various calcium and phosphorus levels. J. Nutr. 121(Suppl. 11):S99–106. doi:10.1093/jn/121.suppl_11.S99

Hill, R. C., C. F. Burrows, G. W. Ellison, and J. E. Bauer. 2001. The effect of texturized vegetable protein from soy on nutrient digestibility compared to beef in cannulated dogs. J. Anim. Sci. 79:2162–2171. doi:10.2527/2001.7982162x

Kastenmayer, P., G. L. Czarnecki-Maulden, and W. King. 2002. Mineral and trace element absorption from dry dog food by dogs, determined using stable isotopes. J. Nutr. 132(6 Suppl. 2):1670S–1672S. doi:10.1093/jn/132.6.1670S

Kim, M., D. T. Lee, and Y.K. Lee. 1995. Iron absorption and intestinal solubility in rats are influenced by dietary proteins. Nutr. Res. 15:1705–1716. doi:10.1016/0271-5317(95)02041-0

Myers, W. D., P. A. Ludden, V. Nayigihugu, and B. W. Hess. 2004. Technical note: a procedure for the preparation and quantitative analysis of samples for titanium dioxide. Journal of Animal Science. 82:179–183. doi:10.2527/2004.821179x.

Propst, E. L., E. A. Flickinger, L. L. Bauer, N. R. Merchen, and G. C. Fahey, Jr. 2003. A dose-response experiment evaluating the effects of oligofructose and inulin on nutrient digestibility, stool quality, and fecal protein catabolites in healthy adult dogs. J. Anim. Sci. 81:3057–3066. doi:10.2527/2003.81123057x

Purushotham, B., P. M. Radhakrishna, and B. S. Sherigara. 2007. Effects of steam conditioning and extrusion temperature on some anti-nutritional factors of Soyabean (Glycine max) for pet food applications. Am. J. Anim. Vet. Sci. 2:1–5. doi:10.3844/ajavsp.2007.1.5.

Rehman, Z., and W. H. Shah. 2005. Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. Food Chem. 91:327–331. doi:10.1016/j.foodchem.2004.06.019

Schmitt, S., J. Mack, E. Kienzle, L. G. Alexander, P. J. Morris, A. Colyer, and B. Dobenecker. 2018. Faecal calcium excretion does not decrease during long-term feeding of a low-calcium diet in adult dogs. J. Anim. Physiol. Anim. Nutr. (Berl.) 102:e798–e805. doi:10.1111/jpn.12837

Southgate, D. A. T. 1989. The role of the gut microflora in the digestion of starches and sugars: with special reference to their role in the metabolism of the host, including energy and vitamin metabolism. In: Dietary starches and sugars in man: a comparison. London: Springer. p. 67–87. Available from https://link.springer.com/chapter/10.1007/978-1-4471-1701-8_4

Stein, H. H., O. Adeola, G. L. Cromwell, S. W. Kim, D. C. Mahan, and P. S. Miller, North Central Coordinating Committee on Swine Nutrition (NCCC-42). 2011. Concentration of dietary calcium supplied by calcium carbonate does not affect the apparent total tract digestibility of calcium, but decreases digestibility of phosphorus by growing pigs. J. Anim. Sci. 89:2139–2144. doi:10.2527/jas.2010-3522

Swanson, K. S., C. M. Grieshop, E. A. Flickinger, L. L. Bauer, J. Chow, B. W. Wolf, K. A. Garleb, and G. C. Fahey, Jr. 2002a. Fructooligosaccharides and lactobacillus acidophilus modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. J. Nutr. 132:3721–3731. doi:10.1093/jn/132.12.3721

Swanson, K. S., C. M. Grieshop, E. A. Flickinger, L. L. Bauer, H. P. Healy, K. A. Dawson, N. R. Merchen, and G. C. Fahey, Jr. 2002b. Supplemental fructooligosaccharides and mannanoligosaccharides influence immune function, ileal and total tract nutrient digestibilities, microbial populations and concentrations of protein catabolites in the large bowel of dogs. J. Nutr. 132:980–989. doi:10.1093/jn/132.5.980

Topper, K., and J. Kotuby-Amacher. 1990. Evaluation of a closed vessel acid digestion method for plant analyses using inductively coupled plasma spectrometry. Commun. Soil Sci. Plant Anal. 21:1437–1455. doi:10.1080/00103629009368315

US EPA, O. 2015. SW-846 Test Method 3051A: Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils. US EPA. Available from https://www.epa.gov/hw-sw846/sw-846-test-method-3051a-microwave-assisted-acid-digestion-sediments-sludges-soils-and-oils

Weremko, D., H. Fandrejewski, T. Zebrowska, I. K. Han, J. H. Kim, and W. T. Cho. 1997. Bioavailability of phosphorus in feeds of plant origin for pigs – review. Asian-Austr. J. Anim. Sci. 10:551–566. doi:1997.10.6.551