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Apparent total tract digestibility, fecal characteristics, and blood parameters of healthy adult dogs fed high-fat diets

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Abstract

Pet foods may be formulated with decreased starch to meet consumer demands for less processed diets. Fats and oils may be added to low-starch diets to meet energy requirements, but little is known about its effects on canine health. The study objective was to evaluate the effects of feeding healthy adult dogs low carbohydrate, high-fat diets on apparent total tract digestibility, fecal characteristics, and overall health status. Eight adult Beagles were enrolled in a replicated 4 × 4 Latin Square design feeding trial. Dogs were randomly assigned to one of four dietary fat level treatments (T) within each period: 32% (T1), 37% (T2), 42% (T3), and 47% (T4) fat on a dry matter basis. Fat levels were adjusted with the inclusion of canola oil added to a commercial diet. Each dog was fed to exceed its energy requirement based on NRC (2006). Blood samples were analyzed for complete blood counts, chemistry profiles, and canine pancreatic lipase immunoreactivity levels. Apparent total tract digestibility improved ($P < 0.05$) as the fat level increased for dry matter, organic matter, fat, and gross energy. Fecal output decreased as levels of fat increased in the diet ($P = 0.002$). There was no effect of fat level on stool quality or short-chain fatty acid and ammonia concentrations in fecal samples ($P \geq 0.20$). Blood urea nitrogen levels decreased with increased fat level ($P = 0.035$). No significant differences were seen in canine pancreatic lipase immunoreactivity ($P = 0.110$). All blood parameters remained within normal reference intervals. In summary, increased dietary fat improved apparent total tract digestibility, did not alter fecal characteristics, and maintained the health status of all dogs.

Key words: canned food, dogs, fat, health, starch

Introduction

Throughout evolution, dogs have developed the ability to digest and metabolize carbohydrates but do not have a nutrient requirement for them (NRC, 2006). Even without this nutrient requirement, pet foods contain high amounts of carbohydrates to meet energy demands, provide lower cost products, and for processing considerations. Pet owners have developed an interest in the nutrition and dietary ingredients present in pet foods (Buff et al., 2014). Consumers have recently developed a desire to feed their dogs less processed foods as compared to the instinctual diets eaten by their canine ancestors (Morelli et al., 2019). There has also been increased popularity in less processed products such as freeze-dried and raw diets (Buff et al., 2014; Carter et al., 2014; Schlesinger and Joffe, 2011). To create these products, diets may be formulated with a decreased concentration of starches. However,

Abbreviations

ALT	alanine aminotransferase
BCS	body condition score
BUN	blood urea nitrogen
CBC	complete blood count
CP	crude protein
cPLI	canine pancreatic lipase immunoreactivity
DM	dry matter
DMB	dry matter basis
GE	gross energy
MCV	mean corpuscular volume
MCH	mean corpuscular hemoglobin
MCHC	MCH concentration
ME	metabolizable energy
MPV	mean platelet volume
OM	organic matter
RBCs	red blood cells
RDW	red blood cell distribution width
SCFA	short-chain fatty acid
TDF	total dietary fiber
WBCs	white blood cells

with this decrease in carbohydrates, it is still necessary to maintain the energy requirements of the diet with additional ingredients that fulfill this energy demand. Fat is included from 8% to 22% on a dry matter basis (**DMB**) in kibble diets and 20% to 32% (DMB) in canned diets to increase caloric density and improve palatability. Fat is typically not included at higher levels due to difficulties in processing, health concerns, and the fact that current fat levels are already above nutrient requirements (Lin et al., 1998; NRC, 2006). One commonly mentioned health concern is the increased risk of pancreatitis with the consumption of high-fat diets in dogs (Xenoulis et al., 2008). However, little is known about the effects of high-fat diets with low levels of starch on canine health. The study objective was to evaluate the effects of feeding healthy adult dogs increasing levels of fat in low carbohydrate diets on apparent digestibility, fecal characteristics, and overall health status. We hypothesized that increased dietary fat would improve the apparent digestibility of the diet while maintaining fecal characteristics and overall health status of each dog.

Materials and Methods

The protocol for this experiment was reviewed and approved by the Iowa State University Institutional Animal Care and Use Committee before initiation of the experiment (IACUC #9-17-8605-K).

Animals and housing

Eight healthy spayed female Beagles, 1 yr of age with an average baseline bodyweight of 8.57 ± 0.93 kg and body condition score (**BCS**) of 4.75 ± 1.16, according to the Royal Canin BCS chart for small dogs, were enrolled in this study. All dogs were housed in pairs at the College of Veterinary Medicine at Iowa State University in temperature-controlled rooms (20 °C) on a 12:12 (light:dark) h schedule. During feeding and collection periods, dogs were separated by gate closure.

Experimental design

Dogs were randomly assigned to one of four dietary treatments using a replicated 4 × 4 Latin Square design consisting of 15-d periods. This design allowed each dog to receive each diet for one period during each replicate. Each period included a 10-d diet adaption phase followed by a 5-d total collection phase.

Diets and feeding

A commercially manufactured canned canine diet (Table 1) was used as control. Increasing inclusion levels of fat (2%, 4%, or 6% canola oil, as-fed basis) were added to control diet to create three more treatments. Treatment diets contained 32% (T1), 37% (T2), 42% (T3), and 47% (T4) total dietary fat (DMB) (Table 2). Dogs were fed twice daily (0800 hours and 1700 hours) to meet their daily energy requirements. Total daily energy requirements were calculated per treatment for each individual dog based on body weight at the beginning of each period. Weights and BCS were recorded weekly. If needed, feed intake was adjusted during the adaption phase in an attempt to maintain an ideal BCS of 4 according to the Royal Canin BCS chart for small dogs. Water was provided ad libitum throughout the study.

Sample collection

Before the beginning of the trial, a 5-mL sample of blood was collected from each dog via jugular venipuncture to assess complete blood count (**CBC**) and chemistry panels to determine any underlying health concerns that were present and could confound data collection. Fecal samples were also collected and evaluated before the start of the study to ensure all dogs were parasite free.

A 5-mL sample of blood was also collected from each dog via jugular venipuncture on d 15 of each period. The blood samples were split into two collections tubes: one red-top tube and one lavender-top Ethylenediaminetetraacetic acid tube. Samples were submitted to the Clinical Pathology Laboratory at Iowa State University College of Veterinary Medicine (Ames, IA) for a CBC (ADVIA 2120i Hematology System; Siemens Healthcare; Erlangen, Germany), chemistry panel (VITRO 5.1 FS Chemistry Analyzer; Ortho Clinical Diagnostics, Raritan, NJ), and canine pancreatic lipase immunoreactivity (**cPLI**) analysis.

Kennels were checked for feces at least every hour for 24 h during each collection day. Feces were weighed, scored, and stored at −20 °C until laboratory analyses. Fecal output and fecal scores were recorded for each dog during each collection period. Fecal scores were determined using the following scale: 1 = hard dry and crumbly feces to 5 = watery diarrhea (Moxham, 2001). Fresh samples (within 15 min) were collected for short-chain fatty acid (**SCFA**) and ammonia concentrations. pH was

Table 1. Ingredient composition of control diet

Diet	Ingredients
Control	Chicken, chicken broth, chicken liver, carrots, peas, dried egg product, guar gum, carrageenan, ground flaxseed, potassium chloride, salt, cassia gum, minerals (zinc amino acid chelate, iron amino acid chelate, copper amino acid chelate, manganese amino acid chelate, sodium selenite, potassium iodide), vitamins (vitamin E supplement, thiamine mononitrate, niacin supplement, d-calcium pantothenate, vitamin A supplement, riboflavin supplement, biotin, vitamin B12 supplement, pyridoxine hydrochloride, vitamin D3 supplement, folic acid), choline chloride

Table 2. Analyzed chemical composition and estimated ME of diets (DM basis)[1]

	Canola oil			
Item	0%	2%	4%	6%
DM, %	22.15	24.85	24.94	26.74
Moisture, %	77.85	75.15	75.06	73.26
OM, %	88.96	90.74	90.63	91.60
Ash, %	11.05	9.27	9.37	8.41
CP, %	46.88	42.72	40.02	38.19
Fat, %	32.05	37.15	41.86	46.49
Total Dietary Fiber, %	3.41	3.34	3.27	3.20
Total Starch, %	1.08	1.06	1.04	1.02
GE, kcal/kg	6,068.01	6,361.67	6,488.54	6,705.12
ME[2], kcal/kg	4,596.40	4,916.15	5,150.60	5,418.15

[1]All analyses were performed using 2 replicates/diet with a coefficient of variation < 0.5.
[2]ME = 8.5 kcal of ME/g of fat + 3.5 kcal of ME/g of CP + 3.5 kcal of ME/g of nitrogen-free extract.

also determined from this sample. Two milliliters of HCl were added to 2 g of feces and placed in –20 °C for SCFA and ammonia analyses. Two grams of feces were placed into a cryovial tube and immediately stored in –80 °C for microbe analysis.

Chemical analyses

Total fecal collections and dietary treatments were analyzed for macronutrient composition and energy. All chemical analyses were conducted in the Comparative Nutrition Laboratory at Iowa State University (Ames, IA). A subsample (100 g) of each diet was pooled and homogenized. Feces collected during the 5-d collection period were pooled and homogenized for each dog for nutrient analysis. Fecal samples and dietary subsamples were dried at 65 °C in a forced air-drying oven and ground with a coffee grinder to accommodate a small sample size (model BCG11OB; KitchenAid). Diet and fecal samples were analyzed for dry matter (**DM**) (AOAC 934.01) and organic matter (**OM**) (AOAC 942.05). Crude protein (**CP**) was determined using a LECO Nitrogen Analyzer (AOAC 992.15; model TruMacN; LECO Corporation; St. Joseph, MI). An EDTA sample of 9.56% nitrogen was used as the standard for calibration. Crude fat was determined via acid hydrolysis and hexane extraction (AOAC 960.39). Gross energy (**GE**) was determined via bomb calorimetry (model 6200; Parr Instrument Co.; Moline, IL) with benzoic acid (6,318 kcal GE/kg; Parr Instrument Co.) used as the standard for calibration. Total dietary fiber (**TDF**) and starch content were determined with the use of assay kits (Megazyme International, Wicklow, Ireland). Metabolizable energy (**ME**) values were estimated using The Association of American Feed Control Officials modified Atwater equation:

$$\text{ME} = 8.5\text{kcal ME/g of fat} + 3.5\text{kcal/g of CP} + 3.5\text{ kcal/g of nitrogen-free extract}$$

Apparent total tract digestibility and energy calculations

Feed intake was recorded for each dog throughout the experiment. Total fecal output collected daily during the collection phase of each period was averaged to determine daily fecal output (g as-is/d).

Apparent total tract macronutrient and energy digestibility were determined using chemical composition data from diet and fecal samples and feed intake/fecal output records. Apparent total tract macronutrient and GE digestibility was calculated using the following equation:

$$\text{Apparent digestibility (\%)} = \left(\frac{intake - fecal\ output}{intake}\right) \times 100$$

Statistical analysis

All data were analyzed in a linear mixed model as a replicated 4 × 4 Latin Square design including fixed effects of diet and room (i.e., replicate) and random effects of period and animal (PROC MIXED, Version 9.4, SAS Inst., Cary, NC). Baseline biomarker value, initial body weight, and/or initial BCS were used as covariates in the model depending on each specific trait. Orthogonal contrasts were also performed to analyze linear, quadratic, and/or cubic relationships among treatments. A significant effect of diet and/or of orthogonal contrast was considered at $P < 0.05$.

Results and Discussion

Diet and fecal chemical analyses

Nutrient concentrations ranged for DM (22.2% to 26.7%), OM (89.0% to 91.6%), CP (46.9% to 38.2%), Fat (32.1% to 46.5%), TDF (3.41% to 3.20%), total starch (1.08% to 1.02%), and GE (6,068 to 6,705 kcal/kg) between T1 and T4, respectively. With each addition of 2% canola oil, the overall fat content of the diet increased by 5%, ranging from 32% to 47% total dietary fat for T1 and T4, respectively. Diets were originally formulated based on the estimated protein to fat ratios. Protein and fat often account for most of the nutrient composition in canned diets and are an important factor to ensure a well-balanced diet during formulation. The protein to fat ratios of the final diets were 1.46 and 0.82 for T1 and T4 resulting in a shift from the primary macronutrient of protein to fat. While the addition of fat increased the DM, OM, fat, and GE in the diets, it decreased the amount of protein, TDF, and starch. Of note, canola oil is a source of pure fat; therefore, it does not contribute to other nutrients. In addition, canola oil has a high DM percentage of 99% increasing the diet's DM percentage which may impact fecal output and nutrient digestibility.

Fecal DM significantly increased with inclusion of fat ($P = 0.047$) and followed a linear relationship with treatments ($P = 0.008$) (Table 3). An increase in fecal DM content can best be explained by the increase in DM percentage of the diets or the increase in digestibility as fat increased. Fahey et al. (1990) reported a decrease in fecal DM as diets became less digestible. Organic matter, CP, fat, and GE of fecal samples were not different ($P \geq 0.10$).

Feed intake and fecal characteristics

Feed intake and fecal characteristics are presented in Table 4. Feed intake was controlled to exceed at least 10% of each animal's energy requirement, following NRC guidelines for lab animals. Average feed intake on an as-fed basis decreased ($P = 0.001$) from 547.5 to 388.2 g/d with a negative linear relationship ($P < 0.001$) as the dietary fat level increased. However, feed intake on a DM basis and GE intake were similar throughout treatments ($P \geq 0.09$). As levels of dietary fat increased from T1 to T4, feed offered in grams/day were smaller for treatments with greater energy density. Nonetheless, all nutrients were offered and consumed based on NRC (2006) nutrient requirements on a grams/day basis. Dogs also maintained ideal body weight and BCS throughout the trial (Table 5).

Table 3. Chemical composition of fecal samples (DM basis)

	Canola oil					P-value			
Item	0%	2%	4%	6%	SEM	Treatment	Linear	Quadratic	Cubic
DM, %	33.38[b]	34.98[a,b]	35.22[a,b]	36.40[a]	1.38	0.047	0.008	0.773	0.466
OM, %	62.57	63.16	63.79	63.41	0.50	0.208	0.092	0.238	0.557
Ash, %	37.43	36.84	36.21	36.59	0.50	0.208	0.092	0.238	0.557
CP, %	29.49	29.58	29.83	29.74	1.18	0.955	0.644	0.848	0.805
Fat, %	8.59	9.59	9.90	8.99	0.88	0.222	0.473	0.055	0.802
GE, kcal/kg	3,506.06	3,561.34	3,639.02	3,586.61	53.10	0.189	0.103	0.211	0.422

[a,b]Means within a row lacking a common superscript letter are different ($P < 0.05$)

Table 4. Feed intake, fecal output, fecal score, fecal pH, urine pH, and apparent total tract macronutrient and energy digestibility

	Canola oil					P-value			
Item	0%	2%	4%	6%	SEM	Treatment	Linear	Quadratic	Cubic
Intake									
Feed intake (as fed), g/d	547.50[a]	479.33[b]	440.70[b,c]	388.23[c]	28.54	0.001	<0.001	0.726	0.666
Feed intake, g DM/d	121.54	119.35	109.73	103.66	6.89	0.097	0.017	0.721	0.653
GE intake, kcal/d	737.54	759.28	712.02	695.03	43.73	0.566	0.263	0.574	0.520
Output									
Fecal output, g as-is/d	60.38[a]	52.78[a,b]	43.25[b,c]	35.53[c]	6.33	0.002	<0.001	0.987	0.833
Fecal output, g DM/d	19.89[a]	18.08[a,b]	14.81[b,c]	13.55[c]	1.78	0.004	<0.001	0.812	0.513
Fecal score[1]	2.42	2.48	2.33	2.30	0.10	0.429	0.192	0.589	0.393
Fecal pH	6.83	6.93	6.93	6.80	0.10	0.629	0.846	0.206	0.915
Urine pH	7.00	ND	ND	7.25	0.35	0.194	ND	ND	ND
Apparent digestibility									
DM, %	83.61[c]	84.69[b,c]	86.55[a,b]	87.62[a]	1.05	0.021	0.002	0.998	0.694
OM, %	88.48[c]	89.35[b,c]	90.50[a]	91.40[a]	0.76	0.019	0.002	0.983	0.852
CP, %	89.73	89.38	89.93	90.22	0.99	0.891	0.567	0.691	0.741
Fat, %	95.57[c]	96.02[b,c]	96.84[a,b]	97.62[a]	0.43	0.001	<0.001	0.613	0.788
GE, %	90.53[c]	91.43[b,c]	95.01[a]	93.35[a,b]	0.79	0.003	0.003	0.118	0.036

[1] Fecal score determined with the use of the Waltham Faeces Scoring System.
[a-c] Means within a row lacking a common superscript letter are different ($P < 0.05$).

Table 5. Bodyweight and body condition score of dogs

	Canola oil					P-value			
Item	0%	2%	4%	6%	SEM	Treatment	Linear	Quadratic	Cubic
Body weight	7.66	7.50	7.53	7.55	0.18	0.199	0.228	0.111	0.385
Body condition score	3.63	3.56	3.56	3.50	0.24	0.907	0.492	1.000	0.818

Fecal output decreased from T1 to T4 on an as-is ($P = 0.002$) and DM basis ($P = 0.004$) with negative linear responses ($P < 0.001$) as dietary fat increased. The decreased total feed intake followed by the improved digestibly with the increased fat percentage may explain the decrease in total fecal output (Kerr et al., 2012). Fecal scores were similar among treatments with an average of 2.4 indicating a well-formed (normal) stool. The normal stool consistency was surprising due to previous concern of loose stool as a result of high-fat diets (Ballaban-Gil et al., 1998; Hosain et al., 2005; Seo et al., 2007; Liu, 2008; Liu and Wang, 2012). Fecal and urine pH were all within normal limits and remained consistent among diets, suggesting that end products, such as SCFA and ammonia, were not affected by the increase of dietary fat.

SCFA are end products of dietary fiber fermentation (Besten et al., 2013). Overall, there were no significant treatment differences among SCFA for the various diets ($P \geq 0.30$) (Table 6). The average percentage of acetate, butyrate, and propionate throughout treatments was 54.5%, 12.3%, and 27.1%, respectively. Of note, proportions of SCFA were similar to those previously reported by Swanson et al. (2002), Bosch et al. (2009), and Schauf et al. (2018) in dogs. Comparable treatment values may show that the slight fluctuation of fiber and starch levels with increased dietary fat was not enough to affect SCFA production. This indicates that starch fermentation in the hindgut was not altered by the diet. This is important as it is critical to maintain physiological SCFA levels for overall health status as SCFA are the main energy source for colonocytes, with butyrate

Table 6. Ammonia and volatile fatty acid composition of fecal samples

	Canola oil					P-value			
Item	0%	2%	4%	6%	SEM	Treatment	Linear	Quadratic	Cubic
Ammonia, %	17.02	16.00	15.55	17.17	1.59	0.864	0.998	0.417	0.836
VFA concentration, mmol/g									
Acetate	30.77	30.29	30.30	29.59	2.82	0.981	0.699	0.957	0.893
Propionate	15.06	15.46	13.90	15.52	1.44	0.670	0.966	0.565	0.282
Butyrate	7.04	7.01	6.93	6.47	0.82	0.932	0.572	0.767	0.915
Isobutyrate	1.05	1.31	0.98	1.10	0.21	0.612	0.833	0.696	0.215
Isovalerate	2.03	2.19	1.91	2.12	0.28	0.883	0.996	0.935	0.433
Valerate	0.31	0.34	0.23	0.18	0.08	0.211	0.059	0.546	0.469
VFA molar proportion, %[1]									
Acetate	54.91	53.68	55.45	54.05	1.03	0.612	0.861	0.937	0.195
Propionate	26.76	27.25	26.13	28.07	0.76	0.301	0.394	0.320	0.162
Butyrate	12.43	12.41	12.51	11.73	0.66	0.827	0.513	0.573	0.738
Isobutyrate	1.75	2.23	1.85	1.94	0.28	0.564	0.858	0.444	0.243
Isovalerate	3.57	3.81	3.65	3.88	0.40	0.902	0.604	0.973	0.598
Valerate	0.58	0.62	0.40	0.33	0.15	0.205	0.060	0.569	0.407

[1]Calculated as the individual VFA concentration / total VFA concentration × 100%.

accounting for up to 70% of total energy consumption (Roediger, 1980, 1982). In addition, SCFA production has a significant role in gut homeostasis (Thorburn et al., 2014) and can limit the growth of pathogenic species by decreasing luminal pH (Swanson et al., 2002). The production of SCFA also promotes a favorable luminal environment for protective bacterial species, including lactic acid bacteria. The maintenance of normal SCFA levels, as it relates to previous studies cited, indicates that the dietary alteration in this study did not impact the production level of SCFA.

Ammonia and BCFA percentages remained similar among treatments ($P \geq 0.20$) (Table 6). The average percent ammonia among all treatments was 16.44%. The average percentage of all treatments for isobutyrate, isovalerate, and valerate was 1.9%, 3.7%, and 0.5%, respectively. Similar ammonia and BCFA levels were also found by Hesta et al. (2003), Barry et al. (2009), and Herstad et al. (2017) in dogs fed varying diets. In agreement with Schauf et al. (2018), fecal ammonia and BCFA were not affected by a high-fat, low-starch diet in dogs. Ammonia and BCFA are putrefactive compounds produced from unutilized amino acids (Kerr et al., 2012), if increased they may have detrimental effects on intestinal and host health (Swanson et al., 2002). Comparable results among treatments may imply that the protein content of each diet was similar enough to maintain consistent fermentation products.

Apparent total tract digestibility

Changes were observed in nutrient digestibility ($P \leq 0.02$) except for CP with a linear increase among treatments for DM, OM, fat, and GE ($P \leq 0.003$) as fat increased (Table 4). There was also a cubic relationship for GE ($P = 0.036$), possibly indicating a point of optimal energy digestibility. Finally, consumption of T4 led

Table 7. Plasma complete blood count

	Canola oil					P-value				
Item	0%	2%	4%	6%	SEM	Treatment	Linear	Quadratic	Cubic	Reference interval[1]
WBCs, $\times 10^3$/uL	7.20	7.62	7.38	6.41	0.48	0.275	0.201	0.135	0.980	6.0 to 17.0
Neutrophils, $\times 10^3$/uL	4.16	4.73	4.62	3.69	0.45	0.307	0.424	0.090	0.950	3.0 to 11.4
Lymphocytes, $\times 10^3$/uL	2.31	2.18	2.15	2.21	0.20	0.936	0.705	0.613	0.973	1.0 to 4.8
Monocytes, $\times 10^3$/uL	0.52	0.37	0.34	0.35	0.07	0.183	0.074	0.209	0.745	0.15 to 1.35
Eosinophils, $\times 10^3$/uL	0.18	0.26	0.26	0.10	0.05	0.075	0.278	0.018	0.743	0.00 to 0.75
Basophils, $\times 10^3$/uL	0.00	0.00	0.00	0.00	0.00	1.000	1.000	1.000	1.000	0.00 to 0.10
RBCs, $\times 10^6$/uL	6.52	6.55	6.56	6.52	0.13	0.994	0.980	0.788	0.951	5.50 to 8.50
Hemoglobin, gm/dL	15.16	15.16	15.23	15.48	0.34	0.830	0.427	0.655	0.920	12.0 to 18.0
Hematocrit, %	45.61	45.63	45.16	46.46	1.11	0.757	0.594	0.464	0.568	37.0 to 55.0
MCV, fL	69.98	69.69	68.93	69.71	0.69	0.617	0.557	0.366	0.445	60.0 to 77.0
MCH, pg	23.28	23.19	23.24	23.19	0.19	0.917	0.653	0.859	0.615	19.5 to 30.0
MCHC, gm/dL	33.24	33.28	33.79	33.28	0.35	0.461	0.621	0.335	0.242	32.0 to 36.0
RDW, %	12.28[b]	12.41[a,b]	12.66[a]	12.19[b]	0.17	0.030	0.980	0.010	0.097	11.6 to 14.8
Platelets, $\times 10^3$/uL	412.75	413.87	415.87	400.00	26.38	0.816	0.537	0.518	0.748	200.0 to 500.0
MPV, fL	9.95	10.50	10.68	10.83	0.43	0.383	0.106	0.593	0.834	7.00 to 11.00

[1]Reference intervals are specific to Iowa State University College of Veterinary Medicine Clinical Pathology Laboratory.
[a,b] Means within a row lacking a common superscript letter are different ($P < 0.05$).

to the greatest digestibility in DM (87.6%), OM (91.4%), and fat (97.6%) compared to other diets.

Overall, the addition of dietary fat increased digestibility. Compared to extruded dry diets, the diets in the present study were similar or higher in digestibility (Castrillo et al., 2001). Specifically, the digestibility of fat exceeded 95% in each treatment. The increase in apparent digestibility with increased levels of dietary fat was to be expected due to the high digestibility of fat. Many studies have reported an increase in digestibility with the addition of dietary fat in swine (Clawson et al., 1962; Lowrey et al., 1962; Greeley et al., 1964; Jorgensen et al., 1992). Clawson et al. (1962) and Greeley et al. (1964) reported that the addition of dietary fat did not affect protein digestibility. Due to microbial fermentation in the large intestine, apparent fecal digestibility may not be an accurate representation of CP digestibility (Hendriks and Sritharan, 2002), which could explain the observed similar results in protein digestibility.

Blood panels

All blood parameters remained within the desired reference intervals indicating healthy animals with only two showing significant differences among treatments (Tables 7 and 8). Red blood cell distribution width (**RDW**) resulted in a difference among treatments (P = 0.030). A quadratic relationship was observed for RDW among treatments (P = 0.010) with values of 12.28%, 12.41%, 12.66%, and 12.19% for T1, T2, T3, and T4, respectively. Measurement of RDW can serve as a possible indicator of cardiovascular disease (Tonelli et al., 2008). However, RDW values remained within reference ranges and were not of clinical significance. Blood urea nitrogen (**BUN**) levels were impacted by diet (P = 0.035), with a linear decrease with the addition of fat (P = 0.005). Even with the decrease in BUN levels from 14.75 to 13.00 mg/dL from T1 to T4, respectively, BUN levels remained within reference intervals even at the highest fat level indicating results were not of clinal significance. As a result of protein metabolism, urea is produced by the liver and is carried by the blood to the kidney for excretion. Therefore, the decrease in total protein intake could have led to fluctuations in BUN levels (Hosten, 1990). In addition to remaining within the desired reference intervals, BUN levels are not a concern in this study because protein requirements were met (g/d) according to NRC (2006) requirements.

In addition to chemistry and complete blood count profiles, cPLI levels were analyzed due to previous concerns of high-fat diets contributing to the development of pancreatitis in dogs (Xenoulis et al., 2008). Pancreatitis is characterized by inflammation of the pancreas when damage to pancreatic tissue occurs as digestive enzymes are activated before release. Currently, serum pancreatic lipase immunoreactivity is the recommended assay for the diagnosis of pancreatitis in dogs since large quantities of pancreatic lipase may enter blood circulation in cases of pancreatitis (Lem et al., 2008). Levels of cPLI for T1, T2, T3, and T4 were 34.63, 44.13, 42.88, and 39.50 µg/L, respectively, with no significant treatment differences (P = 0.110). The normal cPLI levels obtained in this study (≤200 µg/L) indicate that the elevated levels of dietary fat did not result in adverse side effects in the pancreas. The concern for pancreatitis may instead be related to underlying disease such as obesity or have a genetic component which we did not analyze in this study (Hess et al., 1999). Alternatively, pancreatitis is likely a function of an acute ingestion of a high-fat dose from inappropriate consumption rather than a controlled and consistent intake of fat as was fed in this study.

Conclusion

The increase of dietary fat improved digestibility while maintaining fecal characteristics and blood parameters in healthy adult dogs. Further research is needed regarding

Table 8. Serum metabolite and electrolyte concentration

	Canola oil					P-value				
Item	0%	2%	4%	6%	SEM	Treatment	Linear	Quadratic	Cubic	Reference interval[1]
cPLI, µg/L	34.63	44.13	42.88	39.50	4.17	0.110	0.298	0.033	0.498	≤200
BUN, mg/dL	14.75[a]	14.38[a,b]	13.38[b,c]	13.00[c]	0.90	0.035	0.005	1.000	0.529	10.00 to 30.00
Creatinine, mg/dL	0.71	0.75	0.69	0.76	0.04	0.271	0.508	0.525	0.083	0.50 to 1.50
Glucose, mg/dL	70.63	76.13	77.38	74.00	2.29	0.179	0.260	0.058	0.970	68.00 to 115.00
Total Protein, g/dL	5.96	5.80	5.89	5.80	0.10	0.436	0.280	0.646	0.252	5.20 to 7.10
Albumin, g/dL	3.30	3.18	3.24	3.25	0.08	0.546	0.748	0.268	0.388	2.70 to 4.00
Alkaline Phosphatase, IU/L	30.38	33.75	36.50	33.63	3.64	0.570	0.366	0.314	0.715	20.00 to 150.00
ALT, IU/L	69.88	51.75	70.25	59.75	15.63	0.420	0.769	0.674	0.117	24.00 to 90.00
Total Bilirubin, mg/dL	0.20	0.14	0.17	0.13	0.04	0.360	0.173	0.782	0.266	0.10 to 0.60
Cholesterol, mg/dL	186.38	183.75	183.25	183.75	7.89	0.982	0.756	0.795	0.967	132.00 to 300.00
Triglycerides, mg/dL	29.38	30.13	27.50	28.75	1.90	0.772	0.586	0.892	0.383	24.00 to 115.00
Sodium, mEq/L	144.73	144.37	143.87	143.87	0.76	0.616	0.214	0.738	0.783	141.00 to 151.00
Potassium, mEq/L	4.83	4.78	4.90	4.90	0.08	0.423	0.222	0.691	0.292	3.90 to 5.3
Chloride, mEq/L	114.38	114.75	115.00	113.63	0.66	0.208	0.347	0.075	0.478	112.00 to 121.00
Bicarbonate, mEq/L	23.00	22.13	21.88	23.13	0.61	0.268	0.958	0.057	0.713	19.00 to 25.00
Calcium, mg/dL	10.39	10.28	10.40	10.31	0.10	0.468	0.730	0.847	0.132	9.70 to 11.30
Phosphorus, mg/dL	3.98	4.05	4.35	4.14	0.20	0.123	0.124	0.205	0.149	3.20 to 6.00
Magnesium, mg/dL	1.96	1.93	1.94	2.02	0.04	0.301	0.247	0.135	0.827	1.70 to 2.50

[1]Reference intervals are specific to Iowa State University College of Veterinary Medicine Clinical Pathology Laboratory.
[a-c] Means within a row lacking a common superscript letter are different (P < 0.05).

the optimum and maximum inclusion level of dietary fat in canine diets. The practicality of high dietary fat also needs to be investigated as it pertains to pet food processing. Of note, the goal of this study was to investigate the use of high levels of dietary fat in an ideal situation, with the use of healthy adult dogs, to observe if dogs could utilize the high-fat content and maintain health. However, further research is needed to determine the effect of increased dietary fat in broader populations such as with the use of different breeds, senior, diseased, and/or overweight dogs.

Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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