

Effects of inulin or yeast cell-wall extract on nutrient digestibility, fecal fermentative end-product concentrations, and blood metabolite concentrations in adult dogs fed raw meat-based diets

Alison N. Beloshapka, MS; Laura M. Duclos, PhD; Brittany M. Vester Boler, PhD; Kelly S. Swanson, PhD

Objective—To determine the effects of raw meat-based diets with and without inulin or yeast cell-wall (YCW) extract on macronutrient digestibility, blood cell counts, serum metabolite concentrations, and fecal fermentative end-product concentrations in healthy adult dogs.

Animals—6 healthy adult spayed female dogs (mean \pm SD age, 5.5 \pm 0.5 years; mean body weight, 8.5 \pm 0.5 kg).

Procedures—Dogs were fed each of the following 6 diets for 21 days, the order of which was randomly assigned in a Latin square design: beef control, beef and 1.4% inulin, beef and 1.4% YCW extract, chicken control, chicken and 1.4% inulin, and chicken and 1.4% YCW extract. Each diet trial consisted of a phase for diet adaptation (days 0 to 14) and a phase for measurement of urine and fecal output and content (days 15 to 20). On day 21, food was withheld for blood sample collection. Afterward, the next diet trial began immediately.

Results—All dogs maintained desirable fecal quality characteristics and produced low fecal volume. All diets were highly digestible (protein digestibility > 88%; fat digestibility > 97%). Differences in fermentative end-product concentrations among all diets were minor, but a significant increase in fecal short-chain fatty acid concentrations was evident when dogs were fed beef-based diets with inulin and YCW extract. Fecal spermine concentrations were higher with diets containing inulin and YCW extract than with control diets. Blood cell counts and serum metabolite values were within reference limits after each trial. All diets resulted in maintenance of nitrogen balance.

Conclusions and Clinical Relevance—Results suggested the raw meat-based diets evaluated were highly digestible in dogs. The increase in fecal short-chain fatty acid concentrations achieved when inulin and YCW extract were included may be beneficial to canine health. (*Am J Vet Res* 2012;73:1016–1023)

Feeding of raw meat-based diets to pets continues to increase in popularity.¹ Raw meat-based diets, as with other diets, have potential benefits and adverse effects. Reasons for feeding this type of diet include the lack of preservatives and possible improvement of feces, skin, or coat quality¹ as well as the high digestibility reported for domestic cats.^{2–4} Conversely, consumption of raw meat-based diets can increase pathogen expo-

Received February 25, 2011.
Accepted June 8, 2011.

From the Department of Animal Sciences (Beloshapka, Vester Boler, Swanson) and Division of Nutritional Sciences (Swanson), College of Agricultural, Consumer and Environmental Sciences, University of Illinois, Urbana, IL 61801; and Nature's Variety Inc, 6200 N 56th St, Lincoln, NE 68504 (Duclos).

Supported by Nature's Variety Inc.

Presented as an oral presentation at the American Academy of Veterinary Nutrition Meeting, Anaheim, Calif, June 2010, and at the European Society of Veterinary and Comparative Nutrition Meeting, Zürich, September 2010.

Address correspondence to Dr. Swanson (ksswanso@illinois.edu).

ABBREVIATIONS

BCFA	Branched-chain fatty acid
BW	Body weight
CP	Crude protein
DM	Dry matter
DMB	Dry-matter basis
SCFA	Short-chain fatty acid
scFOS	Short-chain fructooligosaccharide
TDF	Total dietary fiber
YCW	Yeast cell wall

sure or cause nutritional imbalances in pets and feeding and storage can be inconvenient to pet owners.^{1,5–8} To reduce health hazards to pet owners and their companion animals, the US FDA Center for Veterinary Medicine has recommended specific guidelines be followed by manufacturers and users of raw meat-based diets.⁹ However, a lack of controlled trials to evaluate the effects of such diets in dogs persists.

Fructans are a group of fermentable carbohydrates classified as prebiotics. To qualify as a prebiotic, a compound must be resistant to gastric acidity, enzymatic hydrolysis, and gastrointestinal absorption (nondigestible); be fermented by cecal or colonic microflora; and selectively stimulate the growth or activity of bacteria that contribute to colonic and host health.^{10–12} Inulin is a long-chain fructan derived from chicory root extract. Mammalian enzymes are unable to break it down; therefore, inulin reaches the colon where it is fermented by intestinal microbes. Inulin has the properties of a prebiotic; however, nearly all research into the effects of inulin in dogs has involved use of an extruded kibble matrix and not a raw meat-based diet matrix.

Yeast cell-wall extracts are moderately fermentable substrates containing a mixture of carbohydrates and proteins that stimulate immune function and protect against infection with certain pathogens in healthy adult dogs.^{13a} Yeast cell walls are rich in mannans, which are believed to prevent adherence of bacteria expressing type 1 fimbriae to the intestinal wall.^{14,15} Additionally, YCW extract may decrease the production of putrefactive compounds such as phenols and indoles when fed in combination with fructans.¹⁶ The effects of this extract when used in raw meat-based diets have yet to be evaluated in dogs.

The purpose of the study reported here was to evaluate the effects of raw meat-based diets, including diets formulated with and without inulin and YCW extract, on healthy adult dogs. The inclusion of fermentable substrates in high-protein, raw meat-based diets that are highly digestible may promote gastrointestinal health by providing fecal bulk or positive fermentative profiles, such as an increase in SCFA concentrations or decrease in protein putrefactive compound concentrations. Specific effects of interest were apparent macronutrient digestibility, fecal characteristics, fecal fermentative end products, blood cell counts, serum metabolite concentrations, and nitrogen balance. We hypothesized that all diets would be highly digestible (DM digestibility > 85%) and maintain nitrogen balance, with inclusion of inulin or YCW extract resulting in an increase in fecal SCFA concentrations and decrease in fecal phenol and indole concentrations.

Materials and Methods

Animals—Six healthy spayed female adult Beagles (mean \pm SD age, 5.5 \pm 0.5 years; mean BW, 8.5 \pm 0.5 kg) were used in the study. All animal care and study procedures were approved by the University of Illinois Institutional Animal Care and Use Committee prior to animal experimentation.

Diet protocol—A 3 \times 2 factorial Latin square design was used, by which each of 6 diets was evaluated: beef control, beef and 1.4% inulin^b (DMB), beef and 1.4% YCW extract^c (DMB), chicken control, chicken and 1.4% inulin (DMB), and chicken and 1.4% YCW extract (DMB). Diets were blended at a commercial facility^d and were comprised of meats and organs procured from animals that passed USDA inspection. They were formulated to contain approximately 25% to 30% CP and 45% to 50% fat on a DMB. Ad libitum access to fresh water was provided throughout the study.

Each 21-day feeding trial consisted of a diet adaptation phase (days 0 to 14) and total and fresh urine and fecal output collection phase (days 15 to 20), during both of which dogs were fed the assigned diet. On day 21, food was withheld for blood sample collection. Afterward, the next trial began immediately, without a washout period.

During the first 12 days of each adaptation phase, dogs were housed individually in runs (1.0 \times 2.1 \times 1.8 m). Two days prior to and during collection days 15 to 20, dogs were housed individually in stainless steel cages (0.9 \times 0.9 \times 0.8 m). On the basis of the maintenance metabolic energy requirement for adult dogs (132 kcal \times BW^{0.75}), information from previous feeding records, and the estimated nutrient digestibility of the diets by the manufacturer, an amount of raw food to maintain BW was offered and intake was measured twice daily (8 AM and 5 PM). Dogs were weighed and body condition was assessed (9-point scale) prior to the 8 AM feeding on every Monday (days 4 and 11) during adaptation and on the first and last day of the sample collection periods.

Sample collection and evaluation—Two days prior to and during each 5-day collection period, dogs received gel capsules containing chromic oxide (0.5 g, q 12 h), which served as a digestibility marker, immediately prior to consuming their food ration. During the 5-day collection phase, all feces were collected, including 1 sample of freshly eliminated (within 15 minutes after elimination) feces/dog/trial. Fresh fecal samples were prepared for analysis immediately to minimize loss of volatile components. All feces, including the fresh samples, were collected from the bottom of the cage, weighed, scored for consistency, and frozen at -20°C for further analysis.

Fresh fecal samples were weighed, and pH was determined with a pH meter^e equipped with an electrode.^f Samples of fresh feces were used to determine DM content. An aliquot (2 g) of fresh feces was mixed with 5 mL of 2N HCl for measurement of ammonia, SCFA, and BCFA concentrations and stored at -20°C until analyzed. Duplicate aliquots (1 g each) of fresh feces were collected for measurement of phenol and indole concentrations and duplicate aliquots (2 g each) of fresh feces were collected for biogenic amine concentrations. All fecal samples were scored for consistency according to the following system: 1 = hard, dry pellets in a small hard mass; 2 = hard, formed, dry feces that remain firm; 3 = soft, formed, moist feces that retain shape; 4 = soft, unformed feces that assume shape of container; and 5 = watery or liquid feces that can be poured.

All urine was collected from days 15 to 20 in vessels containing 5 mL of 2N HCl for immediate acidification to prevent loss of nitrogen, and the volume was recorded. Portions of the acidified urine samples were placed into smaller polyethylene plastic bottles and stored at 4°C until analysis. A fresh urine sample (nonacidified) was also collected and submitted to a diagnostic laboratory^g for complete urinalysis, including determination of urine specific gravity with a refractometer.^h

On the final day of each feeding trial (day 21), 6 mL of blood was collected via jugular venipuncture for

Table 1—Mean and pooled SEM food intake, fecal characteristics, and total tract apparent macronutrient digestibility of 6 adult Beagles fed raw beef- and raw chicken-based diets with or without (control) the inclusion of inulin or YCW extract.

Variable	Beef diet				Chicken diet			
	Control	Inulin	YCW extract	Pooled SEM	Control	Inulin	YCW extract	Pooled SEM
Food intake								
DM (g/d)	98.9	95.6	103.5	4.91	77.6	83.3	74.3	4.99
Organic matter (g/d)	92.7	90.3	96.8	4.59	71.0	77.1	68.1	4.62
CP (g/d)	24.7	24.7	28.3	1.20	24.8	26.2	23.4	1.57
Fat (g/d)	63.2	61.3	65.2	3.11	39.7	42.8	37.4	2.57
Calories (kcal/d)	737.5	725.9	782.0	36.62	527.5	576.0	511.1	34.47
As-is fecal output (g/d)	28.2	24.7	37.6	3.83	38.0	40.0	37.5	3.29
DMB fecal output (g/d)	12.3 ^A	11.1 ^A	17.1 ^B	1.46	16.0	15.5	14.6	1.88
As-is fecal output/DMB food intake	0.29 ^A	0.25 ^A	0.36 ^B	0.02	0.53	0.48	0.51	0.03
Digestibility (%)								
DM	87.36 ^b	89.30 ^a	86.26 ^a	0.34	77.64	80.14	78.95	0.70
Organic matter	93.28 ^b	94.26 ^a	91.74 ^a	0.22	88.75	89.84	88.52	0.40
CP	91.84 ^b	92.25 ^a	89.95 ^a	0.29	88.59	88.38	88.10	0.46
Acid hydrolyzed fat	97.48	97.81	97.34	0.13	96.68	97.63	97.04	0.29
Energy	94.92 ^b	95.66 ^a	93.99 ^a	0.19	91.78	92.73	91.83	0.37
Fecal consistency score	2.27 ^a	2.34 ^a	2.63 ^b	0.21	1.81	1.76	1.83	0.16
Fecal DM (%)	43.48	43.23	37.30	2.31	43.68	38.61	40.65	2.09
Fecal pH	6.78	6.55	6.63	0.24	6.65 ^a	6.20 ^a	6.16 ^a	0.11

^{a-c}Means within a protein source not sharing a common superscript lowercase letter differ significantly ($P < 0.05$) because of the fiber source.
^{A,B}Means within a protein source not sharing a common superscript uppercase letter differ but not significantly ($P > 0.05$ but ≤ 0.10) because of the fiber source.
Fecal samples were scored for consistency as follows: 1 = hard, dry pellets in a small hard mass; 2 = hard, formed, dry feces that remain firm; 3 = soft, formed, moist feces that retain shape; 4 = soft, unformed feces that assume shape of container; and 5 = watery or liquid feces that can be poured.

blood cell count and serum metabolite measurements. Two milliliters of blood was immediately transferred to an appropriate evacuated tube,ⁱ and the remaining 4 mL of blood was transferred to another evacuated tube.^j Blood samples were transported to the laboratory^g for biochemical analysis.^k

Also on day 21, 2 evaluators scored hair condition (1 = dull, coarse, and dry; 2 = poorly reflective and non-soft; 3 = medium reflective and medium soft; 4 = highly reflective and very soft; 5 = greasy) and skin condition (1 = dry; 2 = slightly dry; 3 = normal; 4 = slightly greasy; 5 = greasy)¹⁷ at the region between the scapulae and at the base of the tail. The evaluators were unaware of which diet the dogs had consumed.

Chemical analyses—Samples of each diet were freeze-dried^l and ground through a 2-mm screen in a laboratory-scale machine for grinding materials.^m Fecal samples were dried at 55°C for 1 week and ground through a 2-mm screen in the grinding machine.^m Diet and fecal samples were analyzed in accordance with the procedures advocated by the Association of Official Analytical Chemists for DM (105°C), organic matter, and ash.¹⁸ Diet, fecal, and urine CP content was calculated from total nitrogen values.¹⁸ Total lipid content (acid-hydrolyzed fat) of the diets and feces was determined in accordance with the methods of the American Association of Cereal Chemists¹⁹ and Budde.²⁰ Gross energy content of diet, fecal, and urine samples was measured by use of an oxygen bomb calorimeter.ⁿ Dietary fiber concentrations (TDF, soluble dietary fiber, and insoluble dietary fiber) were determined.²¹ Samples of all 6 raw meat-based diets were sent to a laboratory^o for complete amino acid profile¹⁸ and mineral content analysis (calcium, phosphorus, zinc, iron, and magnesium)

Table 2—Percentage (as is) of various ingredients in raw chicken- and raw beef-based diets with and without (control diets) the inclusion of inulin (0.56%) or YCW extract (0.56%).

Ingredient	Control	Inulin	YCW extract
Chicken diet			
Chicken (with bone)	49.81	49.81	49.81
Chicken fat	5.64	5.64	5.64
Chicken meat	13.16	13.16	13.16
Chicken heart	10.34	10.34	10.34
Chicken liver	10.34	10.34	10.34
Premix	10.71	10.15	10.15
Beef diet			
Beef	47.46	47.46	47.46
Beef liver	10.34	10.34	10.34
Ground beef bone	6.86	6.86	6.86
Beef heart	11.28	11.28	11.28
Water	11.94	11.94	11.94
Dicalcium phosphate	1.41	1.41	1.41
Premix	10.71	10.15	10.15

Premix included apple (15.2%), carrot (15.2%), butternut squash (15.2%), egg (11.4%), salmon oil (10.9%), broccoli (8.7%), spinach (8.7%), dried kelp (6.5%), alfalfa sprout (2.2%), taurine (2.2%), apple cider vinegar (1.1%), parsley (1.1%), blueberry (1.1%), and various tocopherols (0.5%).

by use of inductively coupled plasma optical emission spectroscopy.²²

To estimate total macronutrient digestibility, chromium concentrations in fecal samples were analyzed as described elsewhere²³ by use of atomic absorption spectrophotometry.^p Fecal short-chain fatty acid and BCFA concentrations were determined via gas chromatography²⁴ by use of a gas chromatograph^q and a glass column^r (180 cm × 4 mm internal diameter). Phenol and indole concentrations were also determined via gas chromatography.²⁵ Ammonia concentrations were determined as described elsewhere.²⁶ Biogenic amine con-

centrations were measured via high-performance liquid chromatography.²⁵

Calculations—Percentage of DM recovery was calculated by dividing chromium intake by chromium concentrations in fecal samples. Total macronutrient digestibility values were calculated as nutrient intake minus fecal output and then divided by nutrient intake multiplied by 100.

The amount of digestible energy was estimated by subtracting the gross energy content of the feces from the gross energy content of the food consumed. The amount of metabolizable energy was determined by subtracting the gross energy content of the feces and urine from the gross energy content of the food consumed.

Statistical analysis—Although the original plan was to assess differences in effects among the 6 diets, differences in food intake between the 2 protein

sources (ie, greater intake in dogs fed beef-based diets) rendered it inappropriate to compare results for beef-based with those of chicken-based diets. Therefore, all statistical analyses were limited to the effects of inulin or YCW extract within a given protein source. A repeated-measures analysis for a crossover study design was conducted for each protein source by use of statistical software.⁸ A procedure for fitting generalized linear mixed models⁴ was used to compare fecal scores and skin and coat condition scores among diets. The experimental unit was considered the dogs. Values of $P < 0.05$ were considered significant.

Results

Animals—Food intake (during 5-day sample collection phase) did not differ within each protein source

Table 3—Nutrient content (DMB) of raw chicken- and raw beef-based diets with or without (control diet) the inclusion of inulin or YCW extract.

Nutrient	Beef diet			Chicken diet		
	Control	Inulin	YCW extract	Control	Inulin	YCW extract
DM (%)	41.43	41.78	42.15	32.61	33.03	32.73
Organic matter (%)	93.70	94.52	93.52	91.41	92.60	91.64
CP (%)	24.99	25.83	24.43	32.00	31.47	31.49
Acid hydrolyzed fat (%)	63.86	64.13	62.97	51.10	51.35	50.30
TDF (%)	3.45	1.01	3.14	4.55	3.53	4.53
Insoluble fiber (%)	3.13	0.98	2.17	3.33	1.64	2.41
Soluble fiber (%)	0.31	0.03	0.97	1.22	1.89	2.12
Gross energy (kcal/g)	7.46	7.60	7.56	6.79	6.92	6.88
ME _{AAFCO} (kcal/g)	6.35	6.48	6.31	5.60	5.68	5.56
ME _c (kcal/g)	6.79	6.96	6.85	5.88	6.03	5.96
Essential amino acids (%)						
Arginine	1.65	1.54	1.46	2.02	1.94	1.98
Histidine	0.64	0.63	0.60	0.81	0.76	0.77
Isoleucine	1.06	1.04	1.00	1.38	1.30	1.32
Leucine	2.00	1.94	1.87	2.40	2.27	2.30
Lysine	1.94	1.90	1.81	2.40	2.32	2.33
Methionine	0.54	0.52	0.48	0.69	0.67	0.67
Phenylalanine	1.09	1.09	1.03	1.30	1.17	1.19
Threonine	0.99	0.89	0.90	1.24	1.20	1.23
Tryptophan	0.27	0.27	0.27	0.32	0.32	0.32
Valine	1.33	1.30	1.27	1.60	1.50	1.56
Nonessential amino acids (%)						
Alanine	1.63	1.54	1.45	1.84	1.77	1.84
Aspartic acid	2.13	2.04	1.93	2.67	2.56	2.59
Cysteine	0.33	0.29	0.27	0.36	0.37	0.36
Glutamic acid	3.08	3.06	2.75	3.85	3.63	3.66
Glycine	2.11	1.89	1.74	2.05	2.01	2.15
Hydroxylysine	0.14	0.09	0.12	0.14	0.10	0.11
Hydroxyproline	0.62	0.47	0.45	0.51	0.53	0.59
Ornithine	0.04	0.05	0.05	0.03	0.03	0.03
Proline	1.43	1.33	1.27	1.49	1.41	1.52
Serine	0.93	0.76	0.78	1.07	1.05	1.08
Taurine	0.13	0.13	0.12	0.21	0.21	0.21
Tyrosine	0.76	0.72	0.76	1.04	0.97	0.96
Minerals						
Calcium	1.12	1.07	1.00	2.05	1.68	2.10
Phosphorus	0.87	0.88	0.86	1.11	0.92	1.18
Iron	0.08	0.06	0.07	0.06	0.05	0.06
Magnesium	0.07	0.05	0.07	0.11	0.10	0.12
Zinc	0.007	0.007	0.007	0.005	0.005	0.005

ME_{AAFCO} = Metabolizable energy determined by use of the Association of American Feed Control Officials method (8.5 kcal of metabolizable energy/g of fat + 3.5 kcal of metabolizable energy/g of CP + 3.5 kcal of metabolizable energy/g of nitrogen-free extract). ME_c = Metabolizable energy determined by use of the following equation: (gross energy intake [kcal/d] – fecal gross energy [kcal/d] – urinary gross energy [kcal/d])/DM intake (g/d).

(beef or chicken), but the 6 Beagles consumed significantly ($P < 0.05$) more of the raw beef-based diets than they ate of the raw chicken-based diets (Table 1).

Diets—Ingredient and nutrient compositions of the 6 raw meat-based diets were summarized (Tables 2 and 3). The composition of the chicken-based diets was similar to the targeted composition, containing a mean of 31.65% CP and 50.92% fat. However, the beef-based diets contained a mean of 25.08% CP and a higher fat content (63.65%) than anticipated. The discrepancy was attributed to the variable composition of the unprocessed raw beef rather than to the processed ingredients. Total dietary fiber content also differed among diets, with the inulin-containing diets containing the lowest amount of TDF. Zinc was present at concentrations slightly lower than what is recommended by the National Research Council²⁷ and the Association of American Feed Control Officials.²⁸

Diet effects—Differences were detected among diets in fecal output, fecal consistency scores, digestibility, and fecal pH. Fecal output (g/d) on a DMB ($P = 0.07$) and fecal output (as is)/food intake (DMB; $P = 0.06$) were greater, albeit not significantly, when dogs were fed the beef and YCW extract diet versus when they were fed the beef control or beef and inulin diets. Compared with the beef control diet, the beef and inulin diet was associated with significantly ($P < 0.05$) higher DM, organic matter, CP, and energy digestibilities but the beef and YCW diet had significantly lower digestibilities. Fecal scores were significantly lower (ie, the feces produced was harder) when dogs were fed the beef control or beef and inulin diets, compared with when fed the beef and YCW diet.

Although fecal output and nutrient digestibility did not differ when dogs were fed chicken-based diets containing inulin or YCW extract, both ingredients contributed to a significantly decreased fecal pH relative to the pH when fed the chicken control diet. The beef-based diets appeared to be more digestible than chicken-based diets, but this difference was not statistically evaluated.

When dogs were fed the beef-based diets, fecal total SCFA and acetate concentrations were significantly greater with the inclusion of inulin or YCW extract (Table 4). Fecal propionate concentrations were numerically but not significantly greater ($P = 0.11$) with the addition of inulin to the beef-based diet. When dogs were fed the chicken-based diets, fecal indole concentration was significantly lower than that of the control diet when inulin or YCW extract was included, whereas fecal total indole and phenol concentrations were significantly lower only with the inclusion of inulin. Fecal spermine concentration was significantly greater with the inclusion of inulin or YCW extract when dogs were fed either protein source. All other fecal fermentative end products were not affected by inulin or YCW extract inclusion.

Mean blood cell counts and serum metabolite concentrations and activities were within reference limits for healthy adult dogs²⁹ throughout the experiment (data not shown), with the exception of serum alanine aminotransferase activity in 1 dog for 1 trial period. Results of urinalysis were unremarkable during all feeding trials. Urine specific gravity did not differ among feeding trials; mean \pm SD urine specific gravity when dogs consumed the beef-based and chicken-based diets was 1.050 ± 0.004 and 1.050 ± 0.003 , respectively. Nitrogen

Table 4—Mean and pooled SEM fecal content values ($\mu\text{mol/g}$) for 6 adult dogs fed raw chicken- and raw beef-based diets with or without (control) the inclusion of inulin or YCW extract.

Variable	Beef diet				Chicken diet			
	Control	Inulin	YCW extract	Pooled SEM	Control	Inulin	YCW extract	Pooled SEM
SCFAs								
Acetate	142.6 ^a	205.3 ^b	189.1 ^b	13.32	150.2	220.3	220.2	21.29
Propionate	45.0	83.0	69.2	10.12	54.1	94.9	79.3	14.92
Butyrate	37.6	42.9	53.8	5.64	32.8	39.3	69.7	12.19
Total SCFAs	225.2 ^a	331.1 ^b	312.1 ^b	23.88	237.1	354.5	369.2	42.34
BCFAs								
Valerate	1.25	1.01	1.19	0.18	0.92	0.72	1.09	0.20
Isovalerate	9.85	10.25	9.21	1.97	7.67	7.50	9.33	1.23
Isobutyrate	6.50	6.47	6.01	1.28	5.06	4.49	5.57	0.73
Total BCFAs	17.60	17.73	16.42	3.20	13.65	12.71	15.99	2.13
Ammonia	125.90	131.10	128.20	12.63	105.14	140.16	108.97	21.94
Phenol	0.43	0.29	0.17	0.20	0.34	0.11	0.15	0.10
Indole	1.56	1.04	0.89	0.21	0.97 ^b	0.37 ^a	0.59 ^a	0.09
Biogenic amines								
Tryptamine	0.32	0.32	0.31	0.03	0.28	0.21	0.36	0.09
Putrescine	2.84	1.79	2.52	0.61	2.2	1.5	1.5	0.33
Cadaverine	0.58	0.33	0.84	0.15	0.45	0.46	0.33	0.21
Tyramine	1.00	0.26	0.93	0.50	0.57	0.25	0.45	0.15
Spermidine	0.89	0.99	1.17	0.11	1.25	1.28	1.53	0.20
Spermine	0.97 ^a	2.70 ^c	1.73 ^b	0.17	1.24 ^a	2.21 ^b	1.82 ^b	0.14
Total biogenic amines	6.62	6.40	7.49	1.13	5.96	5.90	5.95	1.02

Total SCFAs was calculated as acetate + propionate + butyrate. Total BCFAs was calculated as valerate + isovalerate + isobutyrate.
See Table 1 for remainder of key.

balance (retained nitrogen) did not differ among feeding trials (1.06 ± 0.19 g/dL for the beef-based diets and 0.81 ± 0.15 g/dL for the chicken-based diets).

When dogs were fed beef-based diets, the skin condition score in the tail region was significantly lower when inulin was included (2.8) than when nothing (3.1) or YCW extract (2.9) was added (pooled SEM, 0.12). All other skin and coat scores were not affected by diet (data not shown).

Discussion

A considerable dearth of scientific information exists regarding the effects of raw meat-based diet consumption on dogs. Some safety issues pertaining to raw meat-based diets have been investigated; however, the metabolic and physiologic effects on healthy dogs have not been investigated in a controlled environment with controlled dietary formulations.

Many compositional differences have been identified among animal-based protein sources commonly fed to dogs and cats.³⁰⁻³² For instance, in a study³⁰ of the chemical composition and nutrient digestibility of various animal-based ingredients used to manufacture dog food, the CP content ranged from 30.4% to 67.6% and the fat content ranged from 11.6% to 50.7% among protein sources. Given these compositional inconsistencies in raw ingredients, homemade and commercial diets must be carefully formulated and checked regularly to verify that nutrient requirements are being met.

Variability in raw meat-based diets originates from the source and quality of the animal ingredients used (eg, skeletal muscle, organ meats, and by-products). The diets used in the present study were compositionally different between protein sources, with the largest differences observed in CP, fat, and TDF content, but were similar within a protein source. All diets were formulated to contain approximately 45% to 50% fat and 25% to 30% CP. It appeared the beef products used to manufacture the study diets differed in composition from the beef products listed in our diet formulation program. This difference might have been attributable to differences in beef vendors, animal age, or season. The chicken products were not as variable in those factors, and thus the chicken-based diets were closer to the intended composition than were the beef-based diets.

Metabolizable energy values were similar within a protein source but differed between the 2 protein sources. This was expected given that the raw beef-based diets contained a much higher fat percentage than did raw chicken-based diets. Steatorrhea was not noticed; however, given the high fat content of the diets fed, the diets may not be suitable for all dogs. For example, dogs with pancreatitis or obesity may have difficulty digesting this amount of fat or the amount of fat may exacerbate the disease process.

Dietary fiber content is often low in raw meat-based diets.³¹ Although large amounts of fiber are not necessary, its inclusion is important to minimize constipation associated with such diets. In the present study, TDF values were lowest for the diets containing inulin regardless of protein source. Errors in the TDF assay or in diet formulation or mixing may have contributed to these differences. Inulin is a water-soluble fiber and is

consequently not quantified by the TDF assay. Portions of YCW extract may also be unaccounted for with this assay. Overall, the chicken-based diets contained higher TDF values than did the beef-based diets, and these differences may have been due to errors in diet mixing.

Macrominerals and microminerals are often difficult to balance in raw meat-based diets, with calcium and phosphorus content requiring particular attention. A calcium-to-phosphorus ratio of 1:1 to 2:1 is recommended for diets fed to adult dogs.²⁸ However, most skeletal muscle meats contain phosphorus concentrations that are 20 to 30 times as high as calcium concentrations. Because of this, ground bone was included in the beef-based diets and a chicken source containing bone was used in the chicken-based diets.

For the 6 diets evaluated in the present study, calcium-to-phosphorus ratios were within recommendations for adult dogs, but there were some differences among diets. Raw beef-based diets had a lower calcium-to-phosphorus ratio (1.1:1 to 1.3:1) than did the chicken-based diets (1.8:1 to 1.9:1). This difference was primarily attributed to the fact that chicken carcasses containing both bone and muscle meat were used to manufacture the chicken diets, whereas for the beef diets, a predetermined concentration of ground bone blended with beef meat was used. Dietary zinc concentrations were also low in the diets evaluated in the present study. Although no adverse effects were observed in these study dogs, careful diet formulation and mixing remains important.

Variability in diet composition can lead to differences in the amount of food a dog needs to meet its dietary energy requirement. In the present study, food intake data were limited to the 5-day sample collection phase. Because all dogs were fed to maintain their BW throughout the study, differences in food intake were not due to dog preference for 1 particular diet. Food intake, diet composition, and digestibility interact to affect fecal output characteristics such as consistency score, volume, and fermentative end-product concentrations. Given our previous experience feeding raw meat-based diets to domestic cats,^{2,3} we were not surprised to find that fecal output (as is) in the present study was about half of that reported for dogs fed a kibble diet.^{25,33,34} Those studies also involved Beagles, which had mean \pm SD BW of 12.0 ± 1.3 kg,²⁵ 11.3 to 13.4 kg,³³ and 14.4 ± 0.6 kg.³⁴ When a nonfermentable fiber source was included in a raw diet for cats, low fecal consistency scores were observed (2.1/5; hard feces).² When a more fermentable fiber source was included in those diets, ideal fecal scores were observed (3/5; typical feces), suggesting a fermentable fiber source should be included in raw meat-based diets, particularly for cats prone to constipation. High dietary ash content of such diets may also contribute to hard, dry feces. In the present study, however, results were unexpected. All feeding trials, regardless of whether inulin or YCW extract was included, resulted in desirable fecal scores throughout the duration of the study.

Total tract apparent CP and fat digestibilities of the dogs in the present study were similar to findings of previous raw meat studies^{2-4,35} in cats (CP digestibility, 92.9 to 93.9; fat digestibility, 93.9 to 95.5) performed

in the same laboratory. The digestibility of raw meat is much greater than that typical of kibble. Crude protein digestibility is most commonly affected by the variability and sources of protein used in such diets. Total tract CP digestibility can also be affected and misleading because of microbial metabolism of CP in the hindgut. Of the macronutrient digestibilities in the present study, CP digestibility was the most variable and was greater when dogs consumed beef-based diets versus chicken-based diets. Crude protein digestibility has also been reported to differ between diets fed to exotic felids.³ Because the fiber sources and amounts used in the present study were tightly controlled, differences in total tract apparent macronutrient digestibility were likely not due to fiber but to the higher concentration of animal fiber (ie, collagen) in the chicken-based versus beef-based diets, which was also considered in another study.³⁶

Fecal fermentative end-product concentrations can be used to infer the degree of protein and carbohydrate fermentation in the large bowel. Carbohydrate fermentation by colonic microbes primarily produces SCFAs in the large intestine, providing an important energy source for colonocytes. In contrast, an increase in putrefactants, which are largely responsible for fecal odor and negative outcomes on gastrointestinal health such as phenol, indole, and BCFA production, is an indication of protein fermentation occurring in the large intestine. In the present study, fecal acetate and total SCFA concentrations were greater when inulin or YCW extract were included in the raw beef-based diets, compared with when those components were not included, which is in agreement with findings in other *in vitro*¹³ and *in vivo* canine²⁵ studies. Similarly, apparent but nonsignificant changes were detected when dogs were fed raw chicken-based diets. Given that there was no fermentable fiber source added to the control diets, lower SCFA concentrations were expected, as was found in another study³ involving exotic cats. Furthermore, in the present study, fecal pH was lower when inulin or YCW extract was added versus when the control diet was consumed, providing additional evidence of the fermentable nature of these ingredients.

In the present study, fecal phenol and indole concentrations were lower when dogs were fed raw chicken-based diets containing inulin, compared with when dogs were fed the control or YCW-extract diets. Similar changes occurred when dogs were fed raw beef-based diets containing YCW extract; however, the differences were not significant. The diets containing inulin or YCW extract likely had more carbohydrates available for microbial fermentation, allowing those substrates to be fermented by colonic microbes instead of only protein. With the inclusion of fermentable substrates such as inulin in diets, the amount of harmful protein-derived end products can be reduced. Findings in other studies support this conclusion. In a study³⁷ of the effects of feeding scFOS and *Lactobacillus acidophilus* separately or in combination to healthy adult dogs, dogs fed scFOS had lower fecal total phenol concentrations than control dogs fed a sucrose placebo. In another study,¹⁶ the effect of feeding scFOS, mannanoligosaccharides, or both to adult dogs was evaluated. Fecal indole concentrations were 2.44 $\mu\text{mol/g}$ of fecal DM in

dogs fed the control diet and 1.23 $\mu\text{mol/g}$ of fecal DM in dogs fed scFOS, and fecal total phenol and indole concentrations were 3.03 $\mu\text{mol/g}$ of fecal DM and 1.50 $\mu\text{mol/g}$ of fecal DM, respectively.

Improvements in skin and coat condition in dogs are commonly attributed to feeding of raw meat. However, these claims in the past were primarily based on anecdote. In the present study, 2 blinded evaluators scored the dogs' skin and coat condition after each feeding trial but did not detect differences in these characteristics among the diets. It is possible that feeding for a longer duration may be needed to detect any improvement in coat quality.

Results from the present study suggested that raw meat-based diets have high nutrient digestibility in dogs. Fat and protein digestibilities in particular were quite high. Feeding a raw meat-based diet also resulted in acceptable fecal consistency scores and clinically unremarkable blood metabolite concentrations and activities. More research is needed to confirm the data reported here and to evaluate long-term feeding of raw meat-based diets.

-
- a. Hussein HS, Healy HP In vitro fermentation characteristics of mannanoligosaccharides by dogs and cats (abstr), in *Proceedings*. Waltham Symp 2001:80.
 - b. Inulin, Orafit HP, BENEIO Group, Tienen, Belgium.
 - c. YCW extract, Bio-Mos, Alltech Biotechnology, Nicholasville, Ky.
 - d. Nature's Variety Inc, Lincoln, Neb.
 - e. AP10 pH meter, Denver Instrument, Bohemia, NY.
 - f. Beckman Instruments Inc, Fullerton, Calif.
 - g. University of Illinois Veterinary Medicine Diagnostics Laboratory, Urbana, Ill.
 - h. Leica TS METER refractometer, Leica Microsystems Inc, Buffalo, NY.
 - i. No. 367841 BD Vacutainer Plus, BD, Franklin Lakes, NJ.
 - j. No. 367974 BD Vacutainer Plus, BD, Franklin Lakes, NJ.
 - k. Hitachi 911 clinical chemistry analyzer, Roche Diagnostics, Palo Alto, Calif.
 - l. DuraTop Digital Programmer Bulk Tray Dryer, FTS Systems, Stone Ridge, NY.
 - m. Wiley Mill, model 4, Thomas Scientific, Swedesboro, NJ.
 - n. Oxygen bomb calorimeter, model 1261, Parr Instruments, Moline, Ill.
 - o. University of Missouri Experiment Station Chemical Laboratories, Columbia, Mo.
 - p. Atomic absorption spectrophotometer, model 2380, Perkin-Elmer, Norwalk, Conn.
 - q. Hewlett-Packard 5890A series II gas chromatograph, Palo Alto, Calif.
 - r. Glass column (180 cm \times 4 mm internal diameter) packed with 10% SP-1200/1% H₃PO₄ on 80/100+ mesh Chromosorb WAW, Supelco Inc, Bellefonte, Pa.
 - s. Proc MIXED, SAS, version 9.2, SAS Institute Inc, Cary, NC.
 - t. Proc GLIMMIX, SAS, version 9.2, SAS Institute Inc, Cary, NC.
 - u. Kerr KR, Beloshapka AN, Dikeman CL, et al. Nitrogen metabolism of four raw meat diets in domestic cats (abstr), in *Proceedings*. Waltham Int Nutr Sci Symp 2010:97.

References

1. Michel KE. Unconventional diets for dogs and cats. *Vet Clin North Am Small Anim Pract* 2006;36:1269–1281.
2. Kerr KR, Vester Boler BM, Morris C, et al. Apparent total tract energy and macronutrient digestibility and fecal fermentative end-product concentrations of domestic cats fed extruded, raw beef-based, and cooked beef-based diets. *J Anim Sci* 2012;90:515–522.
3. Vester BM, Beloshapka AN, Middelbos IS, et al. Evaluation of

- nutrient digestibility and fecal characteristics of exotic felids fed horse- or beef-based diets: use of the domestic cat as a model for exotic felids. *Zoo Biol* 2010;29:432–448.
4. Vester BM, Burke SL, Liu KJ, et al. Influence of feeding raw or extruded feline diets on nutrient digestibility and nitrogen metabolism of African Wildcats (*Felis lybica*). *Zoo Biol* 2010;29:676–686.
 5. Freeman LM, Michel KE. Timely topics in nutrition: evaluation of raw food for dogs. *J Am Vet Med Assoc* 2001;218:705–709.
 6. LeJeune JT, Hancock DD. Public health concerns associated with feeding raw meat diets to dogs. *J Am Vet Med Assoc* 2001;219:1222–1225.
 7. Weese JS, Rousseau J, Arroyo L. Bacteriological evaluation of commercial canine and feline raw diets. *Can Vet J* 2005;46:513–516.
 8. Michel KE, Willoughby KN, Abood SK, et al. Attitudes of pet owners toward pet foods and feeding management of cats and dogs. *J Am Vet Med Assoc* 2008;233:1699–1703.
 9. US FDA Center for Veterinary Medicine. Guidance for industry No. 122: manufacture and labeling of raw meat foods for companion and captive noncompanion carnivores and omnivores. 2004. Available at: www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm052662.pdf. Accessed Oct 26, 2010.
 10. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota—introducing the concept of prebiotics. *J Nutr* 1995;125:1401–1412.
 11. Gibson GR, Probert HM, Van Loo J, et al. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev* 2004;17:259–275.
 12. Roberfroid MB. Prebiotics: the concept revisited. *J Nutr* 2007;137:830S–837S.
 13. Vickers RJ, Sunvold GD, Kelley RL, et al. Comparison of fermentation of selected fructooligosaccharides and other fiber substrates by canine colonic microflora. *Am J Vet Res* 2001;62:609–615.
 14. Ofek I, Mirelman D, Sharon N. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. *Nature* 1977;265:623–625.
 15. Neeser JR, Koellreutter B, Wuersch P. Oligomannosidetype glycopeptides inhibiting adhesion of *Escherichia coli* strains mediated by type 1 pili: preparation of potent inhibitors from plant glycoproteins. *Infect Immun* 1986;52:428–436.
 16. Swanson KS, Grieshop CM, Flickinger EA, et al. 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* 2002;132:980–989.
 17. Rees CA, Bauer JE, Burkholder WJ, et al. Effects of dietary flax seed and sunflower seed supplementation on normal canine serum polyunsaturated fatty acids and skin and hair coat condition scores. *Vet Dermatol* 2001;12:111–117.
 18. Association of Official Analytical Chemists. *Official methods of analysis*. 17th ed. Gaithersburg, Md: Association of Official Analytical Chemists, 2006.
 19. American Association of Cereal Chemists. *Approved methods*. 8th ed. Saint Paul: American Association of Cereal Chemists, 1983.
 20. Budde EF. The determination of fat in baked biscuit type of dog foods. *J Assoc Off Agric Chem* 1952;35:799–805.
 21. Prosky L, Asp NG, Schweizer TF, et al. Determination of insoluble and soluble dietary fiber in foods and food products: collaborative study. *J Assoc Off Agric Chem* 1992;75:360–367.
 22. Association of Official Analytical Chemists. *Official methods of analysis*. 17th ed. Gaithersburg, Md: Association of Official Analytical Chemists, 2005.
 23. Williams CH, David DJ, Iismaa O. The determination of chromic oxide in feces samples by atomic absorption spectrophotometry. *J Agric Sci* 1962;59:381–385.
 24. Erwin ES, Marco GJ, Emery EM. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. *J Dairy Sci* 1961;44:1768–1771.
 25. Flickinger EA, Schreijen EMWC, Patil AR, et al. Nutrient digestibilities, microbial populations, and protein catabolites as affected by fructan supplementation of dog diets. *J Anim Sci* 2003;81:2008–2018.
 26. Chaney AL, Marbach EP. Modified reagents for determination of urea and ammonia. *Clin Chem* 1962;8:130–132.
 27. National Research Council. *Nutrient requirements for dogs and cats*. Washington, DC: National Academy Press, 2006.
 28. Association of American Feed Control Officials Inc. *AAFCO official publication*. Oxford, Ind: Association of American Feed Control Officials Inc, 2009.
 29. Kahn CM, ed. *Merck veterinary manual*. 9th ed. Whitehouse Station, NJ: Merck & Co, 2005.
 30. Murray SM, Patil AR, Fahey GC Jr, et al. Raw and rendered animal by-products as ingredients in dog diets. *J Anim Sci* 1997;75:2497–2505.
 31. Dust JM, Grieshop CM, Parsons CM, et al. Chemical composition, protein quality, palatability, and digestibility of alternative protein sources for dogs. *J Anim Sci* 2005;83:2414–2422.
 32. Faber TA, Bechtel PJ, Hernet DC, et al. Protein digestibility evaluations of meat and fish substrates using laboratory, avian, and ileally cannulated dog assays. *J Anim Sci* 2010;88:1421–1432.
 33. Diez M, Hornick JL, Baldwin P, et al. The influence of sugar-beet fibre, guar gum and inulin on nutrient digestibility, water consumption and plasma metabolites in healthy Beagle dogs. *Res Vet Sci* 1998;64:91–96.
 34. Rodriguez C, Blanch F, Romano V, et al. Porcine immunoglobulins survival in the intestinal tract of adult dogs and cats fed dry food kibbles containing spray-dried porcine plasma (SDPP) or porcine immunoglobulin concentrate (PIC). *Anim Feed Sci Tech* 2007;139:201–211.
 35. Vester BM, Burke SL, Dikeman CL, et al. Nutrient digestibility and fecal characteristics are different among captive exotic felids fed a beef-based raw diet. *Zoo Biol* 2008;27:126–136.
 36. Eastoe JE, Long JE. The amino-acid composition of processed bones and meat. *J Sci Food Agric* 1960;11:87–92.
 37. Swanson KS, Grieshop CM, Flickinger EA, et al. Fructooligosaccharides and *Lactobacillus acidophilus* modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. *J Nutr* 2002;132:3721–3731.