

Assessment of protein and amino acid concentrations and labeling adequacy of commercial vegetarian diets formulated for dogs and cats

Kayo Kanakubo, BVSc; Andrea J. Fascetti, VMD, PhD; Jennifer A. Larsen, DVM, PhD

Objective—To determine measured crude protein (CP) and amino acid (AA) concentrations and assess labeling adequacy of vegetarian diets formulated for dogs and cats.

Design—Cross-sectional study.

Sample—13 dry and 11 canned vegetarian diets for dogs and cats.

Procedures—Concentrations of CP and AAs were determined for each diet. Values were compared with the Association of American Feed Control Officials (AAFCO) Dog and Cat Food Nutrient Profiles. Product labels were assessed for compliance with AAFCO regulations.

Results—CP concentration (dry-matter basis) ranged from 19.2% to 40.3% (median, 29.8%). Minimum CP concentrations for the specified species and life stage were met by 23 diets; the remaining diet passed appropriate AAFCO feeding trials. Six diets did not meet all AA minimums, compared with the AAFCO nutrient profiles. Of these 6 diets, 1 was below AAFCO minimum requirements in 4 AAs (leucine, methionine, methionine-cystine, and taurine), 2 were below in 3 AAs (methionine, methionine-cystine, and taurine), 2 were below in 2 AAs (lysine and tryptophan), and 1 was below in 1 AA (tryptophan). Only 3 and 8 diets (with and without a statement of calorie content as a requirement, respectively) were compliant with all pet food label regulations established by the AAFCO.

Conclusion and Clinical Relevance—Most diets assessed in this study were not compliant with AAFCO labeling regulations, and there were concerns regarding adequacy of AA content. Manufacturers should ensure regulatory compliance and nutritional adequacy of all diets, and pets fed commercially available vegetarian diets should be monitored and assessed routinely. (*J Am Vet Med Assoc* 2015;247:385–392)

Popularity of vegetarian and vegan diets for humans has increased for ethical, ecological, and health reasons, and this influences pet food choices for some families.^{1,2} In addition, vegetarian diets are often used for veterinary patients with conditions such as hepatic encephalopathy, food allergies, and urate and cystine urolithiasis. However, for several reasons, vegetarian pet foods have been linked to concerns related to nutritional adequacy. Vegetarian protein sources are often poor sources of specific essential vitamins (vitamin D, vitamin A, niacin, and cobalamin), fatty acids (arachidonic acid, docosahexaenoic acid, and eicosapentaenoic acid), and minerals (calcium and potassium).³ In addition, plants are highly variable in protein concentration and provide incomplete AA profiles for meeting the needs of pets. Therefore, vegetarian diets must be appropriately formulated and balanced, including the use of proper supplementation with purified sources of essential AAs when indicated.

Adequate protein and AA intake is an important consideration for both dogs and cats. Cats are more lim-

ABBREVIATIONS

AA	Amino acid
AAFCO	Association of American Feed Control Officials
CP	Crude protein
DM	Dry matter
ME	Metabolizable energy

ited than dogs in their ability to conserve nitrogen and AAs in the face of inadequate dietary intake.⁴ In addition, sulfur-containing AAs (methionine, cystine, and taurine) are found primarily in animal protein. Although it is not used for protein synthesis, taurine is a required dietary nutrient for cats and is important for several physiologic processes, including retinal function, cardiac function, reproduction, and growth.⁵ Taurine is considered conditionally essential for dogs because they have the metabolic capacity to synthesize it when adequate concentrations of sulfur-containing AA precursors (methionine and cysteine) are available, except for specific breeds⁶ and diseases associated with decreased taurine synthesis.⁷ Taurine deficiency has also been identified in dogs fed low-protein diets for extended periods or fed diets limited in sulfur-containing AAs.^{8,9}

Pet foods sold in the United States are regulated by both federal and state laws. Manufacturers are re-

From the Veterinary Medical Teaching Hospital (Kanakubo) and the Department of Molecular Biosciences (Fascetti, Larsen), School of Veterinary Medicine, University of California-Davis, Davis, CA 95616.

Supported by the Center for Companion Animal Health, School of Veterinary Medicine, University of California-Davis.

Address correspondence to Dr. Larsen (jalarsen@vmth.ucdavis.edu).

sponsible for proper formulation and labeling of products to meet requirements set by the US FDA as well as those mandated by each state, many of which have adopted AAFCO model regulations for pet food.¹⁰ Information from the label is often used by pet owners and veterinarians to assess pet foods; therefore, accuracy and compliance with regulations are expected.¹¹ However, to our knowledge, there have been no studies conducted on the incidence of noncompliance of any category of pet foods with AAFCO model regulations, although there is evidence that some diets may provide CP at concentrations below the minimum guaranteed analysis value.¹²

The primary objective of the study reported here was to measure CP and AA concentrations in commercial vegetarian foods formulated for dogs and cats and to compare those values with minimum required concentrations for the intended species and life stage as established by the AAFCO. A secondary objective was to compare the information on pet food labels with required components as established by the AAFCO. We hypothesized that all diets would meet all nutritional and labeling requirements.

Materials and Methods

Sample—Commercially available over-the-counter diets (foods distributed directly to the public without veterinary oversight) consisting of dry and canned products for dogs and cats that were labeled or marketed as vegan or vegetarian and available during June and July 2014 were obtained from local pet stores and online sources. Similarly labeled or marketed veterinary therapeutic dry and canned diets for dogs were obtained from a local veterinary clinic,^a and 1 diet was donated by an employee of the University of California-Davis Veterinary Medical Teaching Hospital.

Procedures—Information from the labels was compared with 9 AAFCO labeling requirements¹³ (product and brand name, species specification, quantity statement, guaranteed analysis, ingredient statement, nutritional adequacy statement, feeding directions, name and address of manufacturer or distributor, and calorie content). The new labeling requirement for inclusion of the calorie content statement on all pet food labels was included in the AAFCO 2014 official publication.¹³ However, the AAFCO recommended in that publication that enforcement be delayed 18 months for new products in development and 3 years for existing products.¹⁴ Therefore, labels were assessed both including and excluding the statement of calorie content as a requirement. Information that was not provided on the label but was required for assessment was obtained from the product website or by contacting the manufacturer.

A sample of each diet was placed in a plastic bag, labeled with a number corresponding to the product, and submitted for analysis; all analytic laboratories were not aware of the commercial source for each sample submitted for analysis.

A sample of each of the canned diets was manually crushed within the plastic bag until a paste consistency was achieved, whereas dry diets were analyzed without any processing. Dry matter values were obtained

by drying representative samples of each diet (20 g of canned diets and 5 g of dry diets) to a constant weight in a vacuum oven at 95° to 100°C.

In addition, 100 g of each canned diet and 50 g of each dry diet were stored in individual containers and frozen at -80°C. These samples were placed into a freeze-drier for 7 days prior to analysis, and canned diets then were manually crushed into a powder to ensure homogeneity. Approximately 5 g of each freeze-dried diet was submitted to a reference laboratory^b for measurement of total nitrogen concentration via a combustion method.¹⁵ This method was not included in the methods cited by the AAFCO¹⁶; however, results of a comparison study¹⁷ with Kjeldahl analysis revealed that the combustion method had improved repeatability and reproducibility for SD ranges. Twenty diets were measured as single samples, and 4 diets were measured as duplicate samples in accordance with the laboratory's standard procedures. The laboratory's acceptable variance was 6.7%, and analytic variation for the 4 duplicate samples was 0.3%. Crude protein content was determined by use of the following equation: CP percentage = nitrogen percentage × 6.25.

For AA analysis, all freeze-dried samples were ground until they could pass through a 2-mm screen (80 mesh). Approximately 10 mg of each ground sample was hydrolyzed in a vacuumed-sealed glass ampule with 2 mL of 6M HCl at 115°C for 24 hours. The hydrolysate was then dried with nitrogen gas, and the resulting residue was reconditioned with lithium hydroxide loading buffer. This solution was filtered by use of a 0.45- μ m polytetrafluorethylene syringe filter. The AA composition was determined in the filtrate by use of a norleucine internal standard with an automated high-performance liquid chromatography AA analyzer^c at the Amino Acid Laboratory at the University of California-Davis, with methods described elsewhere.¹⁸ Cystine and methionine concentrations were determined by use of performic acid oxidation with acid hydrolysis (hydrobromic acid method¹⁹), and tryptophan concentration was determined by use of a method described elsewhere.²⁰ All diets were measured as single samples. In addition to the internal standard used by the laboratory, a reference sample of purified casein was analyzed concurrently with each batch of sample diets; analytic variation was within 5%.

Measured CP and AA concentrations were compared with the minimum requirement in the AAFCO Dog and Cat Food Nutrient Profiles for the intended species and life stage.¹³ Diets formulated for both dogs and cats were compared with the AAFCO food nutrient profiles for cats. Concentrations of CP and AA were corrected for energy density if the diet contained > 4,000 or > 4,500 kcal/kg of DM for canine or feline diets, respectively.²¹ When assessing whether measured concentrations met the minimum values of the AAFCO food nutrient profiles, consideration was given to the allowed analytic variation for CP and lysine (AAFCO does not specify allowable variations for other AAs).¹⁶

Calorie content was obtained from the label or manufacturer; if calorie content was not provided or could not be obtained, it was calculated from the guaranteed analysis. For calculation of calorie content, measured CP and moisture concentrations were

used; modified Atwater values of 3.5 kcal/g for CP and nitrogen-free extract and 8.5 kcal/g for crude fat were used.¹³ Ash concentration was obtained from the label or manufacturer or were estimated by use of the mean value of the ash concentrations measured for diets.

Statistical analysis—A Shapiro-Wilk test was used to confirm data were nonparametric. Spreadsheet software^d was used to calculate descriptive statistics (median and range).

Results

Twenty-four diets were assessed, consisting of 13 dry diets (9 for dogs,^{e-m} 3 for cats,^{n-p} and 1 for both dogs and cats^q) and 11 canned diets (8 for dogs,^{r-y} 2 for cats,^{z,aa} and 1 for both dogs and cats^{bb}). One dry diet for dogs was donated; the other 23 diets were purchased. There were 21 over-the-counter diets for dogs or cats (or both) and 3 veterinary therapeutic diets for dogs. Dry diets represented 9 manufacturers, and canned diets represented 8 manufacturers. There were 2 dry diets that were manufactured at facilities outside the United States.

Only 3 diets (1 dry and 2 canned), including the statement of calorie content as a requirement, and 8 diets (4 dry and 4 canned), excluding the statement of calorie content as a requirement, were compliant with all pet food label regulations as established by the AAFCO. As indicated by the nutritional adequacy statement (or, when not provided, other label information), 14 diets (7 dry and 7 canned) were intended for adult maintenance, 9 diets (5 dry and 4 canned) were intended for all life stages, and 1 diet (dry) was intended for both growth and adult maintenance. Nutritional adequacy for the designated life stage or stages was substantiated by the formulation method to meet the AAFCO Dog and Cat Food Nutrient Profiles for all but 1 diet (1 dry diet for dogs); that diet successfully completed appropriate AAFCO-recognized animal feeding trials.

When label information was compared with the 9 AAFCO requirements, 20 diets (9 dry and 11 canned) met the requirement for product and brand name, 23 diets (13 dry and 10 canned) met the requirement for species specification, 18 diets (7 dry and 11 canned) met the requirement for quantity statement, 17 diets (6 dry and 11 canned) met the requirement for guaranteed analysis, 17 diets (6 dry and 11 canned) met the requirement for ingredient statement, 18 diets (7 dry and 11 canned) met the requirement for nutritional adequacy statement, 12 diets (7 dry and 5 canned) met the requirement for feeding directions, 20 diets (9 dry and 11 canned) met the requirement for name and address of the manufacturer or distributor, and 8 diets (2 dry and 6 canned) met the requirement for statement of calorie content. Of the diets that failed to meet the AAFCO labeling requirements, 4 had the product name outside of the principal display panel, 1 did not have a species-specification statement on the principal display panel, 6 did not have a quantity statement, 4 did not have a guaranteed analysis and 3 did not have an appropriate guaranteed analysis format (terms used and order of items), 5 had misspelled or duplicated words in the ingredient statement and 2 did not have an appropriate

ingredient statement format (ingredients listed under 2 separate headings [ie, composition and additives]), 6 did not have a nutritional adequacy statement, 4 did not have feeding directions, 2 had misspelled words in the feeding directions, 6 did not have frequency or species specifications in the feeding directions, 4 did not have the name and address of the manufacturer, 14 did not have a statement of calorie content, and 2 did not have an appropriate calorie content format (not listed under a heading of calorie content or no information on method of determination). Both diets manufactured outside the United States did not meet 6 of the AAFCO labeling requirements (including not having a statement of calorie content), whereas some of the 22 diets manufactured within the United States did not meet up to 8 of the 9 requirements (including not having a statement of calorie content). Overall, 9 diets (4 dry and 5 canned) had labels with misspelled words.

None of the diets exceeded the maximum moisture percentage as reported on guaranteed analysis. Median measured moisture concentration of the diets was 4.8% (range, 3.3% to 7.8%) for dry diets and 69.9% (range, 61.4% to 74.3%) for canned diets.

Dried eggs were listed as an ingredient in 1 canned diet, whereas the other 23 diets listed only plant-sourced ingredients. Nineteen diets (11 dry and 8 canned) were supplemented with 1 or more AAs: methionine (7 dry and 4 canned), taurine (10 dry and 7 canned), lysine (7 dry and 2 canned), and tryptophan (5 dry and 0 canned); 1 dry diet was supplemented with both cystine and glycine. All 7 diets formulated for cats were supplemented with taurine. Two dry diets included a minimum taurine concentration claim in the guaranteed analysis (which is optional); both of these diets contained taurine in concentrations that exceeded the AAFCO minimum value. However, 1 of the 7 taurine-supplemented diets contained a measured taurine concentration that was 85% of the minimum listed in the guaranteed analysis.

Median measured CP concentration (DM basis) was 29.8% (range, 19.2% to 40.3%) for all diets. Measured CP concentrations were above the minimum requirement for the AAFCO Dog and Cat Food Nutrient Profiles (DM basis or corrected for energy density when necessary) for the intended species and life stage for 23 diets (12 dry and 11 canned). The dry diet for dogs that did not meet the minimum requirement contained 94% of the minimum required value but had completed an AAFCO-recognized animal feeding trial. One additional canned diet for dogs that exceeded 4,000 kcal/kg of DM contained only 91% of the reported minimum CP for the guaranteed analysis on an as-fed basis but met the AAFCO minimum CP on a DM basis when corrected for energy density. All other diets met the reported minimum CP for the guaranteed analysis.

Eighteen diets (10 dry and 8 canned) contained all AAs in concentrations that met or exceeded the minimum values for the AAFCO Dog and Cat Food Nutrient Profiles (DM basis or corrected for energy density when necessary) for the designated life stage (Table 1). Five diets (all for cats; 3 dry and 2 canned) provided 1 or more AAs at concentrations below the AAFCO minimum value. Of these 5 diets, 1 was below the AAFCO

Table 1—The AA concentrations of vegetarian dry and canned diets formulated for dogs and cats and values for the AAFCO Dog and Cat Food Nutrient Profiles.

AA	Median	Range	AAFCO	
			Growth and reproduction (minimum)	Adult maintenance (minimum)
Canine (n = 17)				
Arginine	1.66	1.08–2.83	0.62	0.51
Histidine	0.59	0.40–0.96	0.22	0.18
Isoleucine	1.05	0.84–1.81	0.45	0.37
Leucine	1.88	1.45–4.74	0.72	0.59
Lysine	1.40	0.99–2.47	0.77	0.63
Methionine-cystine	0.85	0.46–3.62	0.53	0.43
Phenylalanine-tyrosine	2.39	1.92–3.90	0.89	0.73
Threonine	1.13	0.90–1.53	0.58	0.48
Tryptophan	0.25	0.18–0.40	0.20	0.16
Valine	1.29	1.01–2.00	0.48	0.39
Taurine	0.19	0.11–0.30	—	—
Feline (n = 7)*				
Arginine	1.85	1.49–2.50	1.25	1.04
Histidine	0.77	0.68–0.88	0.31	0.31
Isoleucine	1.44	1.28–1.58	0.52	0.52
Leucine	3.41	0.43–4.81	1.25	1.25
Lysine	1.46	1.12–2.18	1.20	0.83
Methionine-cystine	1.63	0.59–3.14	1.10	1.10
Methionine†	0.62	0.51–1.32	0.62	0.62
Phenylalanine-tyrosine	3.20	3.00–3.88	0.88	0.88
Phenylalanine	1.89	1.80–2.22	0.42	0.42
Threonine	1.42	1.10–1.60	0.73	0.73
Tryptophan	0.36	0.16–0.41	0.25	0.16
Valine	1.72	1.51–1.80	0.62	0.62
Taurine (extruded)‡	0.18	0.15–0.18	0.10	0.10
Taurine (canned)§	0.12	0.11–0.15	0.20	0.20
Values reported are percentage DM.				
*Includes results for 2 diets formulated for both dogs and cats. †Methionine is the only AA with a maximum allowed value, and only for feline diets (1.5% DM). ‡Values are for 4 extruded diets. §Values are for 3 canned diets.				
— = Not applicable.				

minimum requirements in 4 AAs (leucine, methionine, methionine-cystine, and taurine), 1 was below in 3 AAs (methionine, methionine-cystine, and taurine), 2 were below in 2 AAs (lysine and tryptophan), and 1 was below in 1 AA (tryptophan). An additional canned diet intended for both dogs and cats exceeded the AA minimum values for dogs but was below the minimum values for cats for 3 AAs (methionine, methionine-cystine, and taurine), despite inclusion of dried eggs as an ingredient. All of the canned diets formulated for cats (2 for cats and 1 for both dogs and cats) were below the AAFCO minimum value for taurine; dry diets for cats exceeded this value. Overall, of the diets that contained 1 or more AAs at concentrations below AAFCO minimum values, the AA concentrations ranged from 34% to 98% (median, 82%) of the minimum requirement stated in the AAFCO Dog and Cat Food Nutrient Profile. The 2 diets below the minimum value for lysine (98% and 93% of the minimum requirement) were within the analytic variation (20%) allowed by the AAFCO regulations; lysine was the only AA for which the AAFCO provided an allowance for analytic variation. All other AAs that did not meet the AAFCO minimum requirement exceeded the range of analytic variation provided by the laboratory.

Calorie content was provided on the label for 10 diets (4 dry and 6 canned). Calorie content was obtained from the product website for 2 diets (1 dry and 1

canned) and from the manufacturer (on a volume basis only [can or cup]) for 10 diets (6 dry and 4 canned). Calorie content information could not be obtained for 2 diets (both dry). Calorie content was calculated for 4 canned diets by use of the per-unit calorie content provided by the manufacturer, 4 dry diets by use of the modified Atwater factor and ash content provided by the manufacturer, and 4 dry diets by use of the modified Atwater factors and mean ash content calculated for dry diets (n = 8) for which the ash concentration was measured (5.76% on an as-fed basis). Median calorie content (DM basis) for all 24 diets was 3,758 kcal of ME/kg of diet (range, 2,915 to 4,316 kcal of ME/kg of diet). Median calorie content (DM basis) of the 17 diets for dogs was 3,725 kcal of ME/kg of diet (range, 3,233 to 4,316 kcal of ME/kg of diet) and of the 7 diets for cats or for both cats and dogs was 3,843 kcal of ME/kg of diet (range, 2,915 to 4,050 kcal of ME/kg of diet). One diet (canned maintenance diet for dogs) required adjustments in nutrient concentrations on the basis of the correction for calorie content.

Discussion

One objective for the present study was to assess product labeling by comparing diet labels with the AAFCO model regulations.¹³ Although all pet foods must comply with federal labeling requirements,²²

many states also mandate specific aspects of the label, often by adopting the AAFCO labeling and formulation requirements in full or in part.¹⁰ Despite the fact all 24 diets were sold in most or all states, and even with exclusion of calorie content as a requirement, only 8 diets (including all 3 veterinary therapeutic diets) were compliant with all label regulations as established by the AAFCO.

There are 3 means of substantiating claims that pet foods are complete and balanced, and the label's nutritional adequacy statement must specify which method is used.²³ The first method is to formulate the diet to meet the AAFCO Dog and Cat Food Nutrient Profiles. The second method is to conduct a feeding trial by use of AAFCO-recognized protocols for the specified life stage; in the case of successful completion of an appropriate feeding trial, the pet food is exempt from meeting nutrient profiles. Third, if a food is a member of a nutritionally similar product family for which the designated lead product has successfully completed an AAFCO-recognized feeding trial, the label of the products for that food family may state that AAFCO feeding trials substantiate the claim of complete and balanced and the nutritional adequacy statements are indistinguishable. In both cases, the label will state that the product has passed animal feeding tests. When a product fails to meet 1 of the aforementioned 3 methods and is not clearly labeled on the principal display as a snack, treat, or dietary supplement, the product must contain a statement that indicates "intended for intermittent or supplemental feeding only." One diet in the present study had a nutritional adequacy statement that indicated it had successfully completed AAFCO-recognized animal feeding trials (which we confirmed by contacting the manufacturer) and was assessed as adequately formulated, although the CP concentration was 94% of the AAFCO nutrient profile minimum value; all AA concentrations exceeded the AAFCO minimum values. Of the 6 diets that did not have nutritional adequacy statements, none were labeled snack or treat, and they did not have a statement to indicate that the product was intended for intermittent or supplemental feeding only. Rather, the labels of those 6 diets included wording that indicated that they were intended to be complete and balanced (phrasing such as "100% complete" and "ideal maintenance"), which was inadequate.

The AAFCO Dog and Cat Food Nutrient Profiles provide minimum values for CP and essential AA concentrations (as well as a maximum value for methionine concentration in foods formulated for cats) for pet foods made with complex, nonpurified ingredients and to account for effects of processing and impacts on digestibility. Most (23/24) diets assessed in the present study met guaranteed analysis claims for minimum CP concentration, and most (23/24) diets exceeded CP minimum values for the AAFCO nutrient profiles; however, CP concentration was assessed with *in vitro* methods that provided an estimate of protein content calculated by use of the nitrogen concentration. As such, the calculated CP value provided no information related to protein quality, which is defined by the digestibility of the protein and the pattern and bioavailability of the AAs. It is generally recognized that plant protein

sources have lower digestibility than do animal protein sources²⁴; however, studies^{25,26} of dogs have found equal total digestibility for soy-based protein when the soy product is adequately processed. Both animal and plant protein sources can vary in quality. Although protein digestibility was not assessed in the present study, short-term studies^{27,28} revealed that animal-protein meals differ in their ability to support nitrogen retention in cats, with chicken and fish meals not differing from corn gluten meal, whereas meat meal is superior to corn gluten meal. Because digestibility, AA pattern, and AA bioavailability are not provided on product labels, protein quality cannot be assessed from a pet food's ingredient list or guaranteed analysis regardless of the fact that nutrients may be present in concentrations that satisfy the corresponding AAFCO nutrient profile. Investigators of 1 study²⁹ reported limitations of measured CP concentrations for the assessment of protein quality of pet food as evaluated with feeding trials on growing rats. They reported that the biological variables for assessment of protein quality (including weight gain, feed efficiency, protein efficiency ratio, net protein ratio, and net protein utilization) had poor correlation with measured CP concentrations.²⁹ Furthermore, the sum of essential AA concentrations was not correlated with measured CP concentration or biological variables (protein efficiency ratio and net protein ratio).²⁹

Concentrations and proportions of AAs are arguably more important than is CP concentration per se, and AA bioavailability should also be considered. Dogs and cats differ from many other species in that they have obligatory bile acid conjugation with taurine rather than glycine, which is associated with variable losses of taurine through feces. Effects of intestinal bacteria on taurine loss appear to be substantial^{30,31} and may be exacerbated by dietary factors. Studies^{32,33} have revealed that cats fed canned versus frozen-preserved diets, or diets with soybean versus casein protein, had lower plasma taurine concentrations, even though the diets were equal in taurine content. The negative effect on taurine status appears to be secondary to augmented loss of bile acids through microbial degradation and accelerated cholecystokinin-mediated turnover of bile acids.³⁴ In addition, fiber likely increases taurine losses in the feces by influencing intestinal bacterial populations as well as through other effects on bile acid metabolism.³⁵ In the present study, the 3 diets for dogs that provided methionine-cystine concentrations closest to the AAFCO minimum value (8%, 25%, and 35% above the minimum value) were all canned diets that did not provide additional purified sulfur-containing AAs. In addition, all 3 canned diets for cats were too low in taurine concentration despite supplementation. Because plant-based diets are typically lower in sulfur-containing AAs and higher in fiber, these factors may contribute to an increased risk of taurine deficiency in both dogs and cats fed vegetarian diets, especially canned products and products that do not provide supplemental taurine or its precursors.

Processing of pet foods impacts protein digestibility as well as AA bioavailability. Conditions for ingredient rendering, extrusion cooking, and can retorting include application of heat, moisture, pressure, or

mechanical shear to inactivate food-borne pathogens, increase shelf-life, increase digestibility of certain nutrients (denaturation of protein, gelatinization of starch, and inactivation of trypsin inhibitors in vegetable protein), and promote desirable flavor and texture.³⁶ However, despite these beneficial effects of processing, some nutrients are lost during processing. Nonenzymatic browning of foods during processing as a result of Maillard reactions is considered a major factor that negatively affects the quality of protein. Depending on the exact conditions and nutrients present, variable AA losses occur (especially losses of lysine, methionine, cystine, and tryptophan).³⁷ Concentrations of 3 of the 4 AAs (all but lysine) were too low in some of the diets assessed in the present study.

Two diets for cats, including 1 diet with purified L-lysine in the ingredient list, did not meet the minimum concentration for lysine as per the AAFCO food nutrient profiles, but the values for these 2 diets were within the analytic variation allowed by the AAFCO. A third diet provided lysine at only 1% above the minimum AAFCO value. However, bioavailability is an important consideration. Acid hydrolysis of protein, which is required for the measurement of AAs in food, results in reversion of damaged (unavailable) lysine and falsely increases the estimate of bioavailable lysine. In 1 study,³⁸ measurement of total lysine overestimated by 87% the bioavailable lysine concentration of 20 diets formulated for cats (10 dry and 10 canned). Lysine is commonly the limiting AA in cereals, and the impact of processing on lysine availability together with a limited ability to accurately assess available lysine concentrations with routine methods is of particular concern for commercially available vegetarian pet foods.

Notably, 6 of the 24 diets assessed in the study reported here were inadequate in 1 or more AAs; 3 of these diets were too low in sulfur-containing AAs (methionine, methionine-cystine, and taurine). However, on the basis of the ingredient lists, all 3 of those diets were supplemented with taurine, and 2 of those 3 diets were also supplemented with methionine. This finding is similar to that in a study³⁹ conducted to investigate nutritional adequacy of 2 commercially available vegan diets for cats. The authors of that study³⁹ found that both diets had inadequate concentrations of taurine, methionine, methionine-cystine, arachidonic acid, and pyridoxine. One of the diets had additional deficiencies of CP, arginine, lysine, calcium, phosphorus, vitamin A, niacin, and vitamin B₁₂, despite label claims of nutritional adequacy and the fact that limiting AAs were listed in the ingredient list as additive supplements. Dietary deficiencies in sulfur-containing AAs and lysine could result in decreased food intake, low growth rate, and negative nitrogen balance in both dogs and cats.⁴⁰⁻⁴⁶ Furthermore, dermatitis has been reported in dogs⁴⁷ and cats^{48,49} with methionine and lysine deficiency, and retinal and cardiac dysfunction has been reported in dogs^{8,9} and cats⁵⁰ with taurine deficiency.

Analysis of results of the study reported here indicated problems with compliance with labeling regulations in addition to concerns regarding adequacy of AA concentrations in commercially available vegetarian pet foods. Overall, only 5 of 21 over-the-counter diets, but

all 3 of the veterinary therapeutic diets, met all requirements for labeling and nutritional adequacy (excluding the recently published regulation for a calorie content statement); however, the sample size was small. Another important limitation of this study was that samples were collected at 1 time point and from 1 batch of each product. The samples that were assessed for CP and AA concentrations may not have been representative because of variations in composition for each batch. In addition, although assay variability for both AA and CP analysis was low, substantial variations in results attributable to laboratory methods were possible. Regardless, all nutritional and labeling requirements should be met consistently, and manufacturers are responsible for quality assurance. It may be informative to measure the CP and AA concentrations across numerous batches to assess variation and more accurately determine the deviation from nutritional adequacy and regulatory compliance.

In the present study, we assessed only a limited number of essential nutrients in commercially available vegetarian pet foods. A more thorough evaluation of other essential nutrients is warranted, especially because important inadequacies of other nutrients in vegan pet foods have been reported.³⁹ In addition, there was no assessment of the animals while consuming the diets; evaluation of blood AA concentrations would provide valuable information for assessing the AA adequacy of pet foods.⁵¹ Only 1 diet had a nutritional adequacy statement indicating that it had passed AAFCO feeding trials to substantiate a claim of complete and balanced for the specified life stages. Given that both the present study and a previous report³⁹ documented deficiencies of nutrients that were declared to have been included in purified form, this may be evidence that manufacturing errors occur or that diets are not formulated properly. Veterinary therapeutic diets may be more appropriate options for vegetarian pet foods because all 3 veterinary diets assessed in the study reported here met current nutritional adequacy and labeling requirements, compared with only 5 of 21 over-the-counter diets that met the nutritional adequacy and labeling requirements. In addition, the US FDA provides allowance for the marketing of veterinary therapeutic diets under the presumption that they are used only under the direction of a licensed veterinarian who is providing recommendations for appropriate use of the product and for monitoring of individual patients.⁵² It may be prudent that such monitoring includes measurement of plasma AA and whole blood taurine concentrations as well as routine assessment of general health to more fully evaluate the status of pets eating vegetarian diets. Given the findings of the present study, this may be of even greater importance for dogs and cats eating canned vegetarian diets, in which case regular monitoring of taurine status in particular is strongly recommended. For all animals and regardless of diet, general routine monitoring and assessment are necessary for adequate nutritional evaluation and to enable clinicians to provide recommendations for individual animals.⁵³

- a. Sacramento Animal Hospital, Sacramento, Calif.
- b. UC Davis Analytical Lab, University of California-Davis, Davis, Calif.

- c. Biochrom 30, Biochrom Ltd, Holliston, Mass.
- d. Microsoft Office Excel 2008, Microsoft Corp, Redmond, Wash.
- e. Ami Dog, Ami, Padova, Italy.
- f. Gourmet Fondue Veggie Cheese Burger Flavor, Evolution Diet Pet Food, Saint Paul, Minn.
- g. Incredibly Delicious Gourmet Pasta, Evolution Diet Pet Food, Saint Paul, Minn.
- h. Vegetarian Formula for Dogs, Dick Van Patten's Natural Balance Pet Foods, Pacoima, Calif.
- i. Vegan Garden Medley Adult, Halo Purely for Pets, Tampa, Fla.
- j. Nature's Recipe Healthy Skin Vegetarian Recipe, Big Heart Pet Brands, San Francisco, Calif.
- k. Veterinary Diets HA Hypoallergenic Canine Formula, Néstle Purina, St Louis, Mo.
- l. Veterinary Diet Canine Vegetarian, Royal Canin, Charles, Mo.
- m. V-dog, V-dog Food, Sacramento, Calif.
- n. Ami Cat, Ami, Padova, Italy.
- o. Gourmet Fondue Veggie Cheese Burger Flavor, Evolution Diet Pet Food, Saint Paul, Minn.
- p. Incredibly Delicious Gourmet Pasta, Evolution Diet Pet Food, Saint Paul, Minn.
- q. Vegan, Wysong Corp, Midland, Mich.
- r. AvoDerm Natural Vegetarian Formula, Central Garden and Pet Co, Walnut Creek, Calif.
- s. Vegetable Stew Entrée, Evolution Diet Pet Food, Saint Paul, Minn.
- t. Vegetarian Formula, Dick Van Patten's Natural Balance Pet Foods, Pacoima, Calif.
- u. Vegan Garden Medley for Dogs, Halo, Purely for Pets, Tampa, Fla.
- v. Nature's Recipe Stew Healthy Skin Vegetarian Recipe Cuts in Gray, Big Heart Pet Brands, San Francisco, Calif.
- w. Organic Vegan Formula, PetGuard, Green Cove Springs, Fla.
- x. Vegetarian Feast Dinner, PetGuard, Green Cove Springs, Fla.
- y. Veterinary Diet Canine Vegetarian, Royal Canin, Charles, Mo.
- z. Gourmet Entrée, Evolution Diet Pet Food, Saint Paul, Minn.
- aa. Vegetable Stew Entrée, Evolution Diet Pet Food, Saint Paul, Minn.
- bb. Vegetarian Dinner, Evanger's Dog and Cat Food Co, Wheeling, Ill.

References

1. Wakefield LA, Shofer FS, Michel KE. Evaluation of cats fed vegetarian diets and attitudes of their caregivers. *J Am Vet Med Assoc* 2006;229:70–73.
2. Joshi M, Mehta MK, Sharma SK. Feeding practices and common nutritional deficiency disorders in dogs. *Vet Pract* 2007;8:83–84.
3. Craig WJ, Mangels AR, American Dietetic Association. Position of the American Dietetic Association: vegetarian diets. *J Am Diet Assoc* 2009;109:1266–1282.
4. Rogers QR, Morris JG. Do cats really need more protein? *J Small Anim Pract* 1982;23:521–532.
5. Sturman JA. Taurine in development. *Physiol Rev* 1993;73:119–147.
6. Kittleson MD, Keene B, Pion PD, et al. Results of the multicenter Spaniel trial (MUST): taurine- and carnitine-responsive dilated cardiomyopathy in American Cocker Spaniels with decreased plasma taurine concentration. *J Vet Intern Med* 1997;11:204–211.
7. Sanderson SL, Osborne CA, Lulich JP, et al. Evaluation of urinary carnitine and taurine excretion in 5 cystinuric dogs with carnitine and taurine deficiency. *J Vet Intern Med* 2001;15:94–100.
8. Sanderson SL, Gross KL, Ogburn PN, et al. Effects of dietary fat and L-carnitine on plasma and whole blood taurine concentrations and cardiac function in healthy dogs fed protein-restricted diets. *Am J Vet Res* 2001;62:1616–1623.
9. Fascetti AJ, Reed JR, Rogers QR, et al. Taurine deficiency in dogs with dilated cardiomyopathy: 12 cases (1997–2001). *J Am Vet Med Assoc* 2003;223:1137–1141.
10. Dzanis DA. Understanding regulations affecting pet foods. *Top Companion Anim Med* 2008;23:117–120.
11. WSAVA Global Nutrition Committee. WSAVA Global Nutrition Committee: recommendations on selecting pet foods. Available at: www.wsava.org/sites/default/files/Recommendations%20on%20Selecting%20Pet%20Foods.pdf. Accessed Apr 18, 2015.
12. Hill RC, Choate CJ, Scott KC, et al. Comparison of the guaranteed analysis with the measured nutrient composition of commercial pet foods. *J Am Vet Med Assoc* 2009;234:347–351.
13. Association of American Feed Control Officials. Model regulations for pet food and specialty pet food under the model bill. In: *2014 official publication*. Oxford, Ind: Association of American Feed Control Officials, 2014;136–164.
14. Association of American Feed Control Officials. Recommendation of enforcement dates. In: *2014 official publication*. Oxford, Ind: Association of American Feed Control Officials, 2014;iii.
15. AOAC official method 990.03. Protein (crude) in animal feed, combustion method, chapter 4. In: Horowitz W, Latimer GW Jr, eds. *Official methods of analysis of AOAC International*. 18th ed. Revision 1. Gaithersburg, Md: AOAC International, 2006;30–31.
16. Association of American Feed Control Officials. Analytical variations (AV) based on AAFCO Check Sample Program. In: *2014 official publication*. Oxford, Ind: Association of American Feed Control Officials, 2014;296–298.
17. Sweeney RA. Generic combustion method for determination of crude protein in feeds: collaborative study. *J Assoc Off Anal Chem* 1989;72:770–774.
18. Spitze AR, Wong DL, Rogers QR, et al. Taurine concentrations in animal feed ingredients; cooking influences taurine content. *J Anim Physiol Anim Nutr (Berl)* 2003;87:251–262.
19. AOAC official method 994.12. Chapter 4: amino acids in feeds. In: Horowitz W, Latimer GW Jr, eds. *Official methods of analysis of AOAC International*. 18th ed. Gaithersburg, Md: AOAC International, 2006;9–19.
20. AOAC official method 988.15. Chapter 45: tryptophan in foods and food and feed ingredients. In: Horowitz W, Latimer GW Jr, eds. *Official methods of analysis of AOAC International*. 18th ed. Gaithersburg, Md: AOAC International, 2006;88–89.
21. Association of American Feed Control Officials. Correcting for energy density. In: *2014 official publication*. Oxford, Ind: Association of American Feed Control Officials, 2014;160–161.
22. US FDA. Pet food labels—general. Available at: www.fda.gov/AnimalVeterinary/ResourcesforYou/UCM047113. Accessed Sep 19, 2014.
23. Association of American Feed Control Officials. Regulation PF7. Nutritional adequacy. In: *2014 official publication*. Oxford, Ind: Association of American Feed Control Officials, 2014;142–143.
24. Neirinck K, Istasse L, Gabriel A, et al. Amino acid composition and digestibility of four protein sources for dogs. *J Nutr* 1991;121:564–565.
25. Clapper GM, Grieshop CM, Merchen NR, et al. Ileal and total tract nutrient digestibilities and fecal characteristics of dogs as affected by soybean protein inclusion in dry, extruded diets. *J Anim Sci* 2001;79:1523–1532.
26. Bednar GE, Murray SM, Patil AR, et al. Selected animal and plant protein sources affect nutrient digestibility and fecal characteristics of ileally cannulated dogs. *Arch Tierernahr* 2000;53:127–140.
27. Funaba M, Oka Y, Kobayashi S, et al. Evaluation of meat meal, chicken meal, and corn gluten meal as dietary sources of protein in dry cat food. *Can J Vet Res* 2005;69:299–304.
28. Funaba M, Tanak T, Kaneko M, et al. Fish meal vs. corn gluten meal as a protein source for dry cat food. *J Vet Med Sci* 2001;63:1355–1357.
29. Hegedüs M, Fekete S, Solti L, et al. Assessment of nutritional adequacy of the protein in dog foods by trials on growing rats. *Acta Vet Hung* 1998;46:61–70.
30. Kim SW, Rogers QR, Morris JG. Dietary antibiotics decrease taurine loss in cats fed a canned heat-processed diet. *J Nutr* 1996;126:509–515.
31. Hickman MA, Rogers QR, Morris JG. Effect of processing on fate of dietary [¹⁴C]taurine in cats. *J Nutr* 1990;120:995–1000.
32. Hickman MA, Bruss ML, Morris JG, et al. Dietary protein source (soybean vs. casein) and taurine status affect kinetics of the enterohepatic circulation of taurocholic acid in cats. *J Nutr* 1992;122:1019–1028.
33. Kim SW, Morris JG, Rogers QR. Dietary soybean protein decreases plasma taurine in cats. *J Nutr* 1995;125:2831–2837.
34. Backus RC, Rogers QR, Rosenquist GL, et al. Diets causing taurine depletion in cats substantially elevate postprandial plasma cholecystokinin concentration. *J Nutr* 1995;125:2650–2657.
35. Stratton-Phelps M, Backus RC, Rogers QR, et al. Dietary rice bran decreases plasma and whole-blood taurine in cats. *J Nutr* 2002;132:17455–17475.

36. van Boekel M, Fogliano V, Pellegrini N, et al. A review on the beneficial aspects of food processing. *Mol Nutr Food Res* 2010;54:1215–1247.
37. Hendriks WH, Emmens MM, Trass B, et al. Heat processing changes the protein quality of canned cat foods as measured with a rat bioassay. *J Anim Sci* 1999;77:669–676.
38. Rutherford SM, Rutherford-Markwick KJ, Moughan PJ. Available (ileal digestible reactive) lysine in selected pet foods. *J Agric Food Chem* 2007;55:3517–3522.
39. Gray CM, Sellon RK, Freeman LM. Nutritional adequacy of two vegan diets for cats. *J Am Vet Med Assoc* 2004;225:1670–1675.
40. Burns RA, Milner JA. Sulfur amino acid requirements of immature Beagle dogs. *J Nutr* 1981;111:2117–2124.
41. Blaza SE, Burger IH, Holme DW, et al. Sulfur-containing amino acid requirements of growing dogs. *J Nutr* 1982;112:2033–2042.
42. Hirakawa DA, Baker DH. Lysine requirement of growing puppies fed practical and purified diets. *Nutr Res* 1986;6:527–538.
43. Milner JA. Lysine requirements of the immature dog. *J Nutr* 1981;111:40–45.
44. Rogers QR, Morris JG. Essentiality of amino acids for the growing kitten. *J Nutr* 1979;109:718–723.
45. Morris JG, Rogers QR, O'Donnell JA. Lysine requirement of kittens given purified diets for maximal growth. *J Anim Physiol Anim Nutr (Berl)* 2004;88:113–116.
46. Teeter RG, Baker DH, Corbin JE. Methionine and cystine requirements of the cat. *J Nutr* 1978;108:291–295.
47. Hirakawa DA, Baker DH. Sulfur amino acid nutrition of the growing puppy: determination of dietary requirements for methionine and cystine. *Nutr Res* 1985;5:631–642.
48. Strieker MJ, Werner A, Morris JG, et al. Excess dietary cystine intensifies the adverse effect of a methionine deficiency in the cat. *J Anim Physiol Anim Nutr (Berl)* 2006;90:440–445.
49. Larsen JA, Outerbridge CA, Fascetti AJ, et al. Skin lesions associated with lysine deficiency in kittens are characterized by inflammation. *Int J Appl Res Vet Med* 2014;12:61–66.
50. Burger IH, Barnett KC. The taurine requirement of the adult cat. *J Small Anim Pract* 1982;23:533–537.
51. Zicker S, Rogers QR. Use of plasma amino acid concentrations in the diagnosis of nutritional and metabolic diseases in veterinary medicine, in *Proceedings. IVth Cong Int Soc Anim Clin Biochem* 1990;1–16.
52. US FDA. Draft compliance policy guide: labeling and marketing of nutritional products intended for use to diagnose, cure, mitigate, treat, or prevent diseases in dogs and cats. www.fda.gov/downloads/ICECI/ComplianceManuals/CompliancePolicyGuidanceManual/UCM318761.pdf. Accessed Oct 4, 2014.
53. Freeman L, Becvarova I, Cave N, et al. WSAVA Nutritional Assessment Guidelines. *J Small Anim Pract* 2011;52:385–396.



From this month's AJVR

Electrocardiogram reference intervals for clinically normal wild-born chimpanzees (*Pan troglodytes*)

Rebeca Atencia et al

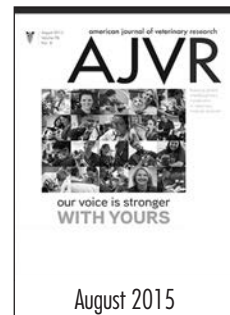
Objective—To generate reference intervals for ECG variables in clinically normal chimpanzees (*Pan troglodytes*).

Animals—100 clinically normal (51 young [< 10 years old] and 49 adult [≥ 10 years old]) wild-born chimpanzees.

Procedures—Electrocardiograms collected between 2009 and 2013 at the Tchimpounga Chimpanzee Rehabilitation Centre were assessed to determine heart rate, PR interval, QRS duration, QT interval, QRS axis, P axis, and T axis. Electrocardiographic characteristics for left ventricular hypertrophy (LVH) and morphology of the ST segment, T wave, and QRS complex were identified. Reference intervals for young and old animals were calculated as mean \pm 1.96•SD for normally distributed data and as 5th to 95th percentiles for data not normally distributed. Differences between age groups were assessed by use of unpaired Student *t* tests.

Results—Reference intervals were generated for young and adult wild-born chimpanzees. Most animals had sinus rhythm with small or normal P wave morphology; 24 of 51 (47%) young chimpanzees and 30 of 49 (61%) adult chimpanzees had evidence of LVH as determined on the basis of criteria for humans.

Conclusions and Clinical Relevance—Cardiac disease has been implicated as the major cause of death in captive chimpanzees. Species-specific ECG reference intervals for chimpanzees may aid in the diagnosis and treatment of animals with, or at risk of developing, heart disease. Chimpanzees with ECG characteristics outside of these intervals should be considered for follow-up assessment and regular cardiac monitoring. (*Am J Vet Res* 2015;76:688–693)



See the midmonth issues
of JAVMA
for the expanded
table of contents
for the AJVR
or log on to
avmajournals.avma.org
for access
to all the abstracts.