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Abstract

Objectives The aim of this study was to analyze iron, copper and zinc concentrations in commercial foods and compare among food formats (dry, canned, raw), foods marketed by age category (adult 1+ years and senior 7+ years) and foods intended for adult maintenance vs all life stages.

Methods In total, 112 commercial non-therapeutic food products marketed for healthy adult and senior cats were purchased in the USA. Foods were analyzed for their proximate composition. Trace mineral concentrations were measured using inductively coupled argon plasma–optical emission spectroscopy and described on a calculated metabolizable energy basis using standard modified Atwater values.

Results Measured iron (median 58.4 mg/1000 kcal [range 15.7–379.0]), copper (median 5.6 mg/1000 kcal [range 0.8–13.3]) and zinc (median 47.6 mg/1000 kcal [range 7.6–128.1]) concentrations were highly variable among cat foods. When all food products – regardless of their nutritional adequacy substantiation method – were compared with the Association of American Feed Control Officials regulatory minimums, 13/112 food products had a mineral deficiency, of which a majority (n = 11/13) were raw food products. Raw foods had significantly lower trace mineral concentrations compared with dry food products and, except for copper, canned food products. Cat foods marketed for senior cats had higher iron ($P = 0.019$) and zinc ($P < 0.0001$) concentrations than foods marketed for adult cats. Foods intended for adult maintenance had higher iron ($P = 0.003$) and zinc concentrations than foods intended for all life stages ($P < 0.0001$).

Conclusions and relevance Iron, copper and zinc concentrations in commercial non-therapeutic foods for adult and senior cats are highly variable. A minority of foods – mainly raw food products – were deficient in these minerals. It is unknown if some foods with high trace mineral concentrations could have adverse effects as studies are needed to establish safe upper limits for dietary intake of trace minerals in healthy cats.

Keywords: Copper; iron; zinc; food; diet; trace minerals

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Introduction

Diet is the main source of the trace minerals copper (Cu), iron (Fe) and zinc (Zn), and adequate dietary intakes of these minerals are essential to maintain normal physiological status and health in cats. These minerals are absorbed by intestinal epithelial cells and transported to tissues for use as cofactors or structural components of proteins that are vital for a variety of physiological functions.[1] In health, the liver, kidney and other tissue are reservoirs for trace minerals.[1,2] Although only trace quantities of these nutrients are needed in the diet, deficient intake of these minerals will, over time, have deleterious effects in cats. Conversely, high intake can reduce bioavailability of other nutrients (eg, Zn interferes with Cu absorption) or might lead to accumulation in tissues of cats, especially the kidney and liver.[3–6]

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The adverse effects of Cu, Fe and Zn accumulation in the body is known in some species. Chronic Cu toxicosis results in liver disease in people, and dietary Cu is a risk factor for the development of copper-associated hepatitis in Labrador Retrievers.[7,8] Chronic Fe overload causes organ dysfunction leading to hepatic failure, cardiomyopathy, diabetes mellitus, neurodegenerative disease and kidney injury in people.[9] In dogs, hepatic failure secondary to chronic administration of a Fe supplement or repeated blood transfusions has been reported.[10,11] In people, extremely high Zn intake results in adverse effects associated with Cu deficiency, including cytopenia and impaired immune function.[12] In rats, excess Zn intake results in kidney dysfunction and hypertension.[13] Little information is available regarding mineral tolerances in cats.[14]

The Association of American Feed Control Officials (AAFCO) and the European Pet Food Industry Federation (FEDIAF) provide nutrient concentration guidance for commercial pet foods (see the Appendix in the supplementary material).[15,16] Many authorities in the USA, as well as in Costa Rica and Puerto Rico, have adopted AAFCO model guidelines as part of their states' feed control laws. Manufacturers that sell pet food in these countries are required to include nutritional adequacy statements on labels. The FEDIAF is a trade association that provides guidelines that its members and other manufacturers in Europe follow through self-regulation. Both AAFCO and FEDIAF base their nutrient guidelines at least partly on the data summarized by the National Research Council (NRC), which provides dietary requirements for cats (see the Appendix in the supplementary material).[17] These published guidelines from the AAFCO, FEDIAF and NRC are established based on interpretation of the published literature and expert opinion. Currently, no AAFCO maximums exist for Cu, Fe and Zn in cat food, and the NRC provides a safe upper limit (SUL) only for Zn ($>$600 mg/kg dry matter). The safety range for Cu, Fe and Zn in commercial cat food is expected to be wide for healthy adult cats.

The concentrations of Cu, Zn and Fe in over-the-counter dry dog foods have been measured previously.[18] The concentrations of these minerals in commercial over-the-counter cat foods is unknown. Therefore, the objective of the study was to measure Cu, Fe and Zn concentrations in commercial non-therapeutic cat food marketed for adult and senior cats. The secondary objective was to compare mineral concentrations between food formats (dry, canned and raw foods), marketed age category (adult 1+ and senior 7+ years), and foods intended for adult maintenance and all life stages based on the AAFCO nutritional adequacy statement on the products' label.

Materials and methods

Cat foods

The Cu, Fe and Zn data for this study were from a food analysis performed in two previously published studies that evaluated calcium and phosphorus content in commercial adult and senior cat foods.[19,20] Cat foods were categorized based on the marketing terms for specific life stages on the label's principle display and on the intended use based on being formulated or feeding trialed for specific life stages as indicated by the label's nutritional adequacy statement. Food (dry, canned and raw) marketed for adult and senior cats were purchased from grocery and pet stores in the Fort Collins, CO, USA area. Cat foods marketed for adults (1+) or all life stages were categorized as adult cat foods. All adult cat food products were assessed in stores prior to purchase and grouped according to food format, and an online randomization tool was used to select the final sample population. All senior cat food products were purchased, and, if available, two lot numbers for each product were purchased for analysis.

Adult and senior cat foods were purchased and shipped for analysis in April 2019 and August 2019, respectively. After purchase, food was stored based on the manufacturer recommendation for the product. Foods were thawed, if needed, and aliquoted into coded containers so that laboratory technicians were blinded to the product information. Samples were shipped overnight on ice to a commercial laboratory (Midwest Laboratory, Omaha, NE, USA) for food analysis.

Food analysis

Food samples were homogenized prior to analysis. Moisture, crude fiber, crude protein, crude fat and ash were determined using proximate analysis by previously validated Association of Official Agricultural Chemists (AOAC) official methods.[21] Total Cu, Fe and Zn concentrations were determined using inductively coupled argon plasma-optical emission spectrometry (AOAC 985.01). The lower limit of detection for total Cu, Fe and Zn were 1.0 ppm, 5.0 ppm and 1.0 ppm, respectively. The reported inter-assay coefficient of variance (CV) for the analysis was 10%. Mineral concentrations were converted to mg/1000 kcal on a ME basis using modified Atwater factors applied to concentrations of measured protein, measured fat and carbohydrate by difference as recommended by AAFCO.[16]

Statistical analysis

Adult and senior cat food products were designated as intended for adult maintenance or for both adult maintenance and growth and reproduction (sometimes referred to as all life stages) based on the AAFCO nutritional adequacy statement on the product's label (see the Appendix in the supplementary material). The inclusion of any Fe, Cu and Zn food additives were recorded from the ingredient lists.

Data were analyzed using statistical software (GraphPad Prism version 9.1.0). The percentage difference in trace mineral concentrations between the two lot numbers for the senior cat food products was calculated,

Table 1 Measured iron, copper and zinc concentrations in all cat foods analyzed, in foods marketed for adult (1+ years) or senior (7+ years) cats, in each food format and in Association of American Feed Control Officials (AAFCO) nutritional adequacy claim categories

	Copper (mg/1000kcal ME)	Iron (mg/1000kcal ME)	Zinc (mg/1000kcal ME)
All foods (n = 112)	5.6 (0.8–13.3)	58.4 (15.7–379.0)	47.7 (7.6–128.1)
Adult (n = 81)	5.6 (0.8–13.3)	54.8 (15.7–135.2)[a]	43.6 (7.6–105.6)[a]
Senior (n = 31)	5.7 (3.1–10.5)	64.9 (29.8–379.0)[b]	57.2 (35.6–128.1)[b]
Food format			
Dry (n = 51)	6.0 (3.2–10.5)[a]	61.4 (29.8–104.5)[a]	57.0 (17.2–122.2)[a]
Canned (n = 41)	5.2 (1.4–13.3)	59.3 (24.5–379.0)[a]	43.8 (21.3–128.1)[b]
Raw (n = 20)	3.6 (0.8–8.1)[b]	34.5 (15.7–74.4)[b]	18.7 (7.6–43.2)[c]
AAFCO Nutritional Adequacy Statement claim			
Adult maintenance (n = 62)	5.5 (2.9–10.5)	60.9 (28.2–379.0)[a]	56.1 (23.0–128.1)[a]
All life stages (n = 50)	5.8 (0.8–13.3)	48.1 (15.7–106.3)[b]	35.7 (7.6–76.0)[b]

Data presented as median (range) and are based on calculated calorie content. Columns bearing a different superscript letter in each category (ie, adult and senior cat foods, food format, and AAFCO Nutritional Adequacy Statement claim) were statistically different from each other.
For senior foods, 26/31 were analyzed as two product lot numbers and are presented as an average value
ME = metabolizable energy

and the average for the two lot numbers was used for statistical analysis. Regardless of the method of AAFCO adequacy substantiation (eg, formulated, family product and food trial), measured mineral concentrations were compared with the minimum concentrations recommended by the AAFCO Cat Food Nutrient Profiles for adult maintenance and growth and reproduction on a calorie basis. Considering the inter-assay CV, a food was deemed deficient in a trace mineral when the calculated trace mineral content was >10% below the minimum AAFCO value. The Shapiro–Wilk test was used to test normality of the data. Non-parametric tests were used for group analysis. The measured Fe, Cu and Zn concentrations were compared among food formats (dry, canned, raw) using the Kruskal–Wallis test with Dunn's multiple comparisons test. Mineral concentrations were compared between foods marketed for senior cats and foods for adult cats and between foods intended for adult maintenance and foods intended for all life stages based on the AAFCO nutritional adequacy statement using the Mann–Whitney U-test. Descriptive data are presented as median (range). A P value <0.05 was considered significant.

Results

Cat food descriptions

In total, 112 food products marketed for adult (n = 81) and senior cats (n = 31) were purchased in the following formats: canned (n = 41); dry (n = 51); and raw (n = 20). Two lot numbers were available for the majority (84%; n = 26/31) of senior foods; thus, a total of 138 food samples were analyzed (81 adult cat food samples; 57 senior cat food samples). The percentage difference in Cu, Fe and Zn concentrations between the two lot numbers for senior cat food products was 10%, 7% and 10%, respectively. Measured mineral concentrations (Cu, Fe, Zn) in the cat foods are presented in Table 1. A complete list of products is provided in the supplementary material.

Three products in the dry format category contained extruded kibble as well as freeze-dried raw pieces. Frozen (n = 11) and freeze- or air-dried (n = 9) products comprised the raw food category, and all raw foods were marketed for adult cats. Based on the nutritional adequacy statements on the labels, foods were formulated to meet AAFCO Cat Food Nutrient Profiles for adult maintenance (55/112 foods); were formulated for all life stages (41/112 foods); had undergone feeding trials adult maintenance (7/112 foods); or were established as family members under AAFCO Pet Food Product Families procedures for all life stages (9/112 foods). The main protein source based on the food ingredient list in the majority of foods was poultry (chicken, n = 33; turkey, n = 11; duck, n = 9; poultry byproducts, n = 5; quail, n = 3) followed by beef (n = 12), fish (salmon, n = 12; tuna, n = 4; mackerel, n = 2; unspecified fish, n = 2; sardine, n = 1), pork (n = 5), rabbit (n = 5), lamb (n = 4), venison (n = 3) and unspecified meat byproducts (n = 1).

When all products regardless of their substantiation method were compared with AAFCO regulatory minimums for their respective life stage claim (adult maintenance or all life stages), 13/112 foods had at least one trace mineral deficiency (Table 1). Of the 96 foods formulated to meet the AAFCO Cat Food Nutrient Profile (vs those that underwent feeding trials or were established as product family members), seven had one or more mineral deficiencies; therefore, the majority (89/96 foods) were in compliance with their respective nutritional adequacy statement. Of the 16 foods that were either food trialed or family products, six had one or more mineral deficiencies when compared with the AAFCO Cat Food Nutrient Profiles for the respective life stage claim.

Copper

Most foods contained a Cu additive (87%; n = 97/112), including Cu sulfate, Cu proteinate and Cu amino acid chelate. No foods contained Cu oxide. Three foods (2.6%; 3/112) were deficient in Cu based on the minimum value for adult maintenance (range 0.84–0.91 mg/1000 kcal ME). These three deficient foods did not have a Cu additive listed on the ingredient list and were categorized as raw foods, as foods marketed for adult cats and as foods intended for all life stages (2/3 family products; 1/3 formulated products). Although AAFCO designates a separate minimum value for dry and canned foods for growth and reproduction, no value is specified for raw foods (see the Appendix in the supplementary material). For canned (n = 15) and dry (n = 16) foods intended for all life stages, one dry food (3.2 mg/1000 kcal ME) and one canned food (1.4 mg/1000 kcal ME) were deficient in Cu based on the minimum value for growth and reproduction. These foods had at least one copper additive and were marketed for adult cats.

Dry foods had higher Cu concentrations when compared with raw foods ($P = 0.01$); no difference was found between dry and canned foods ($P = 0.2$) and between raw and canned foods ($P = 0.5$). No difference was found between foods marketed for adult cats and those for senior cats ($P = 0.4$), and between foods intended for adult maintenance and foods intended for all life stages ($P = 0.3$; Table 1).

Iron

Most foods contained a Fe additive (79%; 89/112) including ferrous sulfate, Fe proteinate and Fe amino acid chelate. No food had Fe carbonate or Fe oxide. Three foods (2.7%; n = 3/112) were deficient in Fe (range 15.7–16.9 mg/1000 kcal ME). These three Fe-deficient foods did not have an iron additive listed on the ingredient list, were categorized as raw foods, were marketed for adult cats and were intended for all life stages (all formulated products).

Raw foods had lower Fe concentrations compared with dry and canned foods ($P < 0.0001$). Foods marketed for senior cats had higher Fe concentrations when compared with those for adult cats ($P = 0.019$). Fe concentrations were higher in foods intended for adult maintenance when compared with foods intended for all life stages ($P = 0.003$; Table 1).

Zinc

Most foods (88%; n = 98/112) had at least one Zn additive, including Zn sulfate, Zn proteinate, Zn amino acid chelate and Zn oxide. Of the 98 foods with a Zn additive, 16 contained Zn oxide, a less absorbable form of Zn used as a coloring agent.[16] Eight foods (7%; n = 8/112) were deficient in Zn (range 7.6–16.5 mg/1000 kcal ME). These eight Zn-deficient foods did not have a Zn additive, and all were categorized as raw, as foods marketed for adult cats and as foods intended for all life stages (5/8 family products; 3/8 formulated products). None of the 16 foods with Zn oxide additive had a Zn concentration below the minimum AAFCO value (median 52.5 mg/1000 kcal; range 23.0–128.1).

Zinc concentrations were different between dry, canned and raw foods (dry vs canned, $P = 0.008$; raw vs dry and raw vs canned, $P < 0.0001$). Foods marketed for senior cats had higher Zn concentrations compared with those for adult cats ($P < 0.0001$). Zinc concentrations were higher in foods intended for adult maintenance compared with foods intended for all life stages ($P < 0.0001$; Table 1).

Discussion

We found the mineral content of the cat foods assessed in this study to be highly variable overall. Most foods (n = 99/112), regardless of their substantiation method, met the AAFCO minimums, and a minority of foods (12%; n = 13/112) had at least one trace mineral deficiency based on their respective AAFCO nutritional adequacy statement. No regulatory maximums for cat foods exist for Cu, Fe or Zn due to the scarcity of data available to regulatory agencies regarding SULs of these trace minerals in cats. The NRC suggests the SUL of Zn is above 600 mg/kg diet on a dry matter basis (roughly extrapolated to be above 150 mg/1000 kcal, assuming the diet caloric density is 4000 kcal/kg dry matter) for at least short periods of time.[15] The NRC defines SUL as the maximum concentration of a nutrient that has not been associated with adverse effects, and SULs are reported when data are available.[22] None of the foods included in our study exceeded the NRC SUL for Zn. To date, data are not available to determine the SUL of Cu and Fe in cats, and therefore it is unknown if some foods in our study with trace mineral concentrations in the upper ranges could have adverse effects. To our knowledge, there are no reports of trace mineral deficiency or toxicity secondary to dietary intake in adult cats fed a commercial food product.

All 13 foods with a trace mineral deficiency were marketed for adult cats (ie, no senior cat foods) and the majority (n = 11/13) were raw products. Although the nutritional adequacy of none of these 11 raw foods was substantiated using AAFCO feeding trials, 6/11 foods were from the same raw food manufacturer and were family products based on the AAFCO adequacy statement. A family member product is similar in a limited number of ways to a lead product that was subject to animal feeding tests. The family members must match the lead products' format (canned, dry, etc) and moisture content, and must be similar in dry matter calorie content, as well as concentrations of crude protein, calcium, phosphorus, Zn, lysine, thiamin, potassium and taurine.[3] Although family members or products that have undergone feeding trials are not required by AAFCO to meet the minimum values in the Nutrient Profiles, those foods with measured trace mineral concentrations below the regulatory limit are a concern regardless of method of substantiation.

In addition to the specific mineral deficiencies noted, raw foods had significantly lower trace mineral concentrations overall when compared with dry food products and, except for Cu, canned food products. This finding points to the low number of raw food products with mineral additives. Of the 20 raw cat food products, only six products contained a Fe (n = 5/6), Zn (n = 6/6) and/or Cu (n = 6/6) food additive. Of those 14 raw foods without a mineral additive, all had a Fe (n = 3/14), Cu (n = 3/14) and/or Zn (n = 8/14) deficiency based on the AAFCO minimum value for adult maintenance. In general, raw food products without a mineral additive accounted for most food products with values below the AAFCO minimum for adult maintenance. Pet food manufacturers should be more stringent in ensuring adequate and consistent concentrations of essential nutrients, either by the using ingredients that provide a natural source of trace minerals or by the addition of trace mineral additives, especially when applying the AAFCO product family procedures. In addition, with the high popularity of raw foods, veterinarians should obtain a dietary history for every cat, and diet must be considered if there are clinical signs consistent with trace mineral deficiency.[23,24]

Ingredient sources of minerals in cat foods come from animal and plant ingredients, as well as mineral-containing additives. Depending on the natural ingredients used in a food product, additives containing Fe, Cu and Zn may be added to meet the regulatory minimum for these trace minerals in cat food. As expected, we found that most commercial cat foods (Fe, 79%; Cu, 87%; and Zn, 88% of foods) declared on the label a mineral additive. Some mineral additives added to cat foods are poorly digestible (especially Fe or Cu oxide and Fe carbonate), and according to AAFCO these sources of trace minerals should not be considered in determining the minimum nutrient concentration.[16] The bioavailability of Zn oxide for cats remains unknown but is likely lower than other sources based on data from other species.[25,26] Research is needed to determine the utility of Zn oxide for cats of all life stages.

Homeostasis of the trace minerals Fe, Cu and Zn is regulated to avoid deficiency and toxicity, both of which can lead to life-threatening health conditions. In veterinary medicine, Cu toxicosis is of great concern, especially in dogs. The liver is the preferential site for Cu accumulation, and excess dietary Cu is normally excreted in bile.[22] When Cu homeostasis is disrupted and excretory pathways are overwhelmed, toxicity occurs, resulting in free radical-induced oxidative damage and cellular toxicity.[27] A growing concern is the role commercial pet foods play in Cu accumulation in the liver of both healthy dogs and in those with primary and secondary hepatopathies.[7,18,28,29] In cats, primary Cu-associated hepatopathy and secondary Cu accumulation in other liver diseases has been reported.[30–33] Similar to dogs,[7] Cu can accumulate in the tissue of cats, especially the kidney and liver.[3,4] In addition, the amount of dietary Cu correlated with tissue concentrations in cats and concentrations in the liver of cats reflect dietary intake.[6,34] Therefore, the lack of defined maximum regulatory standards in commercial cat foods raises concern for potentially high dietary intakes with consequential accumulation of these trace minerals. To our knowledge, there are no reports of adverse effects directly related to excess Cu intake or liver accumulation in adult cats, which is the likely reason for no suggested SUL by the NRC. In addition, the role of dietary Cu intake in cats in the development of primary Cu-associated hepatopathy is unknown and causes beyond gene mutations continue to be elucidated.[33] Whether excessive accumulation of trace minerals in the tissues can cause disease in cats remains unknown.

The bioavailability of trace minerals varies between ingredient sources, whether natural meat, plant sources or food additives. We did not evaluate the bioavailability of Fe, Cu and Zn in the commercial cat foods, nor could we determine the proportions of each mineral contributed by the various sources. Several factors, beyond the inherent bioavailability of the additive or natural ingredient, contribute to trace mineral bioavailability. For example, plant-based ingredients containing phytate inhibit Zn and Fe absorption;[2,35,36] increased dietary Zn concentrations reduce absorption of Cu by interfering with Cu uptake in enterocytes;[5] and macro-mineral dietary concentrations (eg, calcium, magnesium and phosphorus) may interfere with trace mineral absorption.[2,37,38] Therefore, deficiency can occur even with apparently adequate dietary intake.[22] Without bioavailability data, the clinical significance of the study findings is unknown; however, deficiencies are still a concern for products that did not meet the AAFCO minimum values.

Our study had limitations. Owing to the large number of adult cat food products available to the consumer, we were only able to subsample from products available for purchase in the Fort Collins, CO, USA area. Therefore, our results are not representative of all cat food products. It should also be noted that the equation used to estimate ME values for Cu, Fe and Zn from analyzed carbohydrate, protein and fat content relies on modified Atwater factors as recommended by the AAFCO.[22] However, this formula has limitations when applied to different types of cat foods with varying digestibility and fiber content, which may lead to underestimated ME values for highly digestible, lower fiber foods and overestimated ME values for less digestible, higher-fiber foods.[22] Lastly, only one lot number for the adult cat foods was analyzed, and potential variability among lot numbers for these products was not evaluated in this study.

Conclusions

The concentrations of the trace minerals Cu, Fe and Zn in commercial over-the-counter cat foods for adult and senior cats are highly variable. This finding might be due to differences in mineral concentrations in natural ingredients, differences in internal standards established by pet food manufacturers and the lack of regulatory maximums for cat foods. Foods intended for adult maintenance had higher Fe and Zn concentrations than foods intended for all life stages. Foods marketed for senior cats had higher Fe and Zn concentrations than foods marketed for adult cats. Some foods, mainly raw food products, were deficient in trace minerals, and raw food products had significantly lower trace mineral concentrations overall compared with dry food products and, except for Cu, canned food products.

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Supplementary material The following files are available online:
Appendix: Current nutritional guidelines for selected nutrients in foods for feline adult maintenance, growth and reproduction, on a calorie basis.
Complete list of adult and senior cat food products included in the study.

Conflict of interest Stacie Summers is a research consultant for Nestlé Purina PetCare. She also participates as a speaker in continuing education events sponsored or organized by Royal Canin and Boehringer Ingelheim. Jennifer A Larsen is an investigator in clinical trials sponsored by Royal Canin and Nestlé Purina PetCare; she develops educational materials for Brief Media, Mark Morris Institute and *Healthy Pet* magazine; and she also participates as a speaker or attendee in continuing education events sponsored or organized by Royal Canin, Nestlé Purina PetCare and Hill's Pet Nutrition. Jonathan Stockman is a consultant for PetCo Pet Supplies, is an investigator in clinical trials sponsored by Royal Canin and has participated in continuing education events organized or sponsored by Royal Canine, Nestlé Purine PetCare and Hill's Pet Nutrition.

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Ethical approval This work did not involve the use of animals and therefore ethical approval was not specifically required for publication in *JFMS*.

Informed consent This work did not involve the use of animals (including cadavers) and therefore informed consent was not required. No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

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