Title
Eleven-Year Interval Analyses of Fish Oil Supplements for Dogs

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Publication Date
2023

Peer reviewed|Thesis/dissertation

Eleven-Year Interval Analyses of Fish Oil Supplements for Dogs

By

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Nutritional Biology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

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2023

Abstract

Fish oil supplements for dogs are used therapeutically for various clinical conditions, as well as to supply essential omega-3 fatty acids during growth and development. However, products may be variable in quality, potency, and stability. The objective of this study was to analyze 11 canine fish oil supplements from two time points (2010 and 2021) for the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), polychlorinated biphenyl (PCB) arochlors, minerals, and vitamins A and D, as well as to compare their compositions to each other and their respective manufacturer labels. The methods for this investigation differed in some cases between 2010 to 2021, as testing was performed at a different laboratory, or a different method was used, or both in the case of vitamin D. One previously analyzed product was discontinued and replaced.

Molybdenum, cadmium, mercury, lead, and PCBs were not detected in any samples. Other minerals were detected in some products, and concentrations were below chronic exposure limits for adult humans. EPA concentrations varied between timepoints, ranging from -66% to +723%, while DHA varied from -55% to +392%. Overall, there were 10 instances of products not meeting label claims for EPA or DHA. In all 7 instances of minimum content claims for vitamins A and D, the analyzed concentrations did not meet the claims. Overall, products were assessed to have low risk for vitamins A and D toxicity when used as recommended but cannot be considered a reliable nutritional source given the variability and inaccurate label claims.

Introduction

In recent years, there has been a surge in interest in companion animal nutrition, both at the industry and consumer level. Consumer demands have largely driven the humanization of pet food and supplement products. Dietary supplements are popular pet products, containing essential and non-essential nutrients that are regulated like any other animal food. Despite not being regulated as medications, supplement products may have overt or implied health claims. The United States Food and Drug Administration's Center for Veterinary Medicine (US FDA CVM) governs the manufacture and distribution of food, food additives, and drugs given to animals.[1,2] However, the appropriate dosing, safety, and efficacy of nutritional supplements are not guaranteed due at least in part to large market size, limited ability of FDA CVM to proactively identify and address concerns, and monitoring and enforcement priorities.

Fatty acid (FA) supplements are available to pet owners and typically contain omega-3 fatty acids, omega-6 fatty acids, or a mixture of both. The omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are provided in most fish oil supplements, and are essential for several physiological mechanisms. Higher doses are often used for therapeutic benefits for dogs and cats, including promotion of normal cardiac function[3], aiding in gastrointestinal health support[4], and supporting neurological development.[5] For example, a fish oil supplement containing EPA and DHA has been shown to improve symptoms of dermatitis and promote healthier coat characteristics.[5]

As ocean fish is the major source of the fatty acids in many of these products, there is potential for inclusion of minerals, both nutritional elements and heavy metals. The accumulation of high

amounts of arsenic, lead, and mercury can lead to negative health effects, such as genomic instability and DNA damage, as a result of poisoning due to their toxicity.[6] The permitted daily exposure (PDE) limits for human adults set by the United States Pharmacopeia (USP)[7] are a guide to avoid toxicity, but these are not intended for animal use. The American Association of Feed Control Officials (AAFCO) and National Research Council (NRC) provide some guidelines for mineral concentration limits which are intended for animal use.[8,9] For five essential minerals examined in this study (manganese (Mn), selenium (Se), iron (Fe), copper (Cu), and zinc (Zn)), the AAFCO Dog Food Nutrient Profiles Based on Dry Matter includes minimum concentrations for adult maintenance in addition to a maximum limit for Se, while the NRC provides adequate intake and recommend allowance values but no safe upper limits.[8]

Other possible contaminants of concern include polychlorinated biphenyls (PCBs), which are man-made organic chlorinated hydrocarbons, widely used in manufacturing electrical equipment, hydraulic systems, thermal insulation, and plastics.[10] While no longer produced or used in the US, environmental contamination remains a source of most exposure. In fact, fish oil supplements manufactured at plants containing older machinery are at risk, with the FDA acknowledging a tolerance of "2 parts per million in animal feed components of animal origin, including fishmeal and other by-products of marine origin and in finished animal feed concentrates, [and] supplements".[1] One study in 2010 examined higher levels of PCBs in human fish oil supplements containing large fish (salmon and tuna) compared to significantly lower levels in human fish-oil supplements containing small cold-water fish, but similar screening data from pet products has not reported.[11]

Vitamin A and vitamin D are two essential nutrients which are sometimes added to fatty acid supplements or are present as a naturally occurring component of the product. While vitamin A and D supplementation in humans has known benefits, such as relief from an autoimmune disease[12], individuals already consuming a vitamin-fortified diet, individuals could be exposed to high intakes and possible adverse effects. Studies in the late 20th century examined the effects of these vitamins in fish oil supplements on different human life stages, such as pregnancy, and specific effects on canine metabolism.[13]

The objective of this study was to analyze 11 canine fish oil supplements from two time points (2010 and 2021) for nutrients of concern (EPA, DHA, essential minerals, and vitamins A and D) and for potential contaminants (PCB arochlors and heavy metals), as well as to compare their compositions to each other and their respective manufacturer labels.

Methods

Eleven fish oil supplements marketed for pets were collected in both 2010 and 2021, and underwent analyses for fatty acid profile, minerals, PCB arochlors, and vitamins A and D.[a-n] The product selection and purchasing, as well as the preparation of the samples remained the same at each of the two time points. Of the 11 products collected at the first time point, only one could not be obtained at the second time point due to temporary discontinuation and was replaced with another brand that was similar. In 2010, two different lots of nine of the 11 products were – the average was taken for each claimed and reported value for comparison purposes with their 2021 counterpart. Samples from 2021 only came from one lot each. All samples arrived packaged in either liquid or capsule form and contained oil from specific

("Norwegian Sardines," "Salmon Oil," etc.) or nonspecific fish types ("Fish Oil," "Cold Water Fish") as the primary ingredient. The primary ingredients in the 2010 sample group were as follows: 3 salmon oil, 5 mixed fish oil, and 3 fish/vegetable oil combinations. The primary ingredients in the 2021 sample group were as follows: 1 salmon oil, 8 mixed fish oil, and 2 fish/vegetable oil combinations. Overall, 6 samples had changed their ingredients between 2010 and 2021: two samples had changed their primary source of fish-based oil, two samples added mixed tocopherols, one sample added a mineral, and one sample added vitamin A and vitamin D. These additions were taken into consideration for the respective samples' analyzed results for fatty acid, mineral, and vitamin concentrations.

Of the 11 products tested in 2010, two made a claim for vitamin A content on the labels. In 2021, only three of 11 tested product made a minimum claim for vitamin A content on the label. Of the 11 products tested in 2010, only one made a claim for vitamin D on the label. In 2021, only one of 11 tested products made a minimum claim for vitamin D content on the label; two additional products contained added vitamin D according to the ingredient label but made no minimum claims.

Due to the availability from online sellers, products arrived at different times during a 1-month period in their original packaging and were stored in a cabinet away from light at approximately 20°C. At both time points, liquid samples were gently mixed in their original containers, and capsule samples were broken open and emptied into sterile sample containers to ensure homogeneity, as well as to omit any contributions from the capsule itself. The sample weights (averaging 13.72 grams), colors, and consistencies were recorded, and a code was assigned to each sample from 1-11 to mask identity. Glass amber vials were used for the 2010 samples

while sterile plastic tubes were used for the 2021 samples. Once prepared, samples were stored in a freezer approximately -15°C and away from light for about two weeks until sent to the laboratories for analysis.

The fatty acid profiles were analyzed by the Diagnostic Center for Population and Animal Health in 2010 (Lansing, Michigan USA) and by Medallion Labs in 2021 (Minneapolis, WI). At both time points, lipids in each sample were extracted by conventional Mojonnier techniques; the resulting residues were trans-esterified to form fatty acid methyl esters (FAMEs), and finally quantified using capillary column by gas chromatography with flame ionization detection (FID). Total fat, saturated fat, monounsaturated fat, polyunsaturated fat, trans-fat, and individual fatty acids were reported for each sample (mg/mL).

At both time points, mineral contents were analyzed by the California Animal Health & Food Safety Laboratory (CAHFS Laboratory, Davis, CA) using the same methodology. Samples were analyzed for 10 minerals: Mn, Se, Fe, Cu, and Zn as well as arsenic (As), molybdenum (Mo), cadmium (Cd), mercury (Hg), and lead (Pb). Each sample was mixed with a combination of nitric acid and hydrochloric acid, digested at 190°C for 90 minutes, diluted with water, and analyzed using an inductively coupled argon plasma-mass spectrometry (ICP-MS). Laboratory reporting limits were as follows: 0.050 parts per million (ppm) for Mn, As, Se, Mo, Cd, Hg, and Pb, 0.500 ppm for Fe, 0.70 ppm for Cu, and 0.80 ppm for Zn. The detection limits were different among the different analytes to account for the ICP-MS's sensitivity to the lowest routinely quantified concentration of the specific analyte within each sample. Results were reported in ppm, then converted to µg/g for unit consistency during data analysis.

At both time points, PCB arochlors were analyzed at the CAHFS Laboratory using the same methodology. Each sample was dissolved in 5% ethanol acetate and cleaned using gel permeation chromatography (GPC) on a column containing S-X3 Bio-Beads. The GPC eluant was then concentrated, the solvent exchanged with toluene, and analyzed for arochlors 1221, 1232, 1242, 1248, 1254, 1260, and 1262 using gas chromatography with electron capture detection (GC-ECD). A 1.0 part per million (ppm) detection limit was set by the laboratory for all 2010 samples and for seven 2021 samples, while a 10.0 ppm limit was set for the remaining four 2021 samples. These four samples had produced an increase in the baseline signal of the GC-ECD used for analysis; to account for this, these samples were diluted by a factor of 10 which impacted reporting limits but not overall concentrations. Final results were reported in ppm.

Vitamin A was analyzed at Eurofins Scientific, Inc. in 2010 (Petaluma, CA USA) and the CAHFS Laboratory in 2021, using the same methodology. Potassium hydroxide in 80% ethanol was added, and samples were saponified overnight at room temperature. The vitamin A was then extracted from the resulting solution using petroleum ether, concentrated, then exchanged to methanol. This methanol solvent was then run through high-performance liquid chromatography with fluorescent detection (HPLC-FD) to analyze each sample's concentration of vitamin A. A 2.5 ppm detection limit was set which accounts for the lowest routinely quantified concentration of an analyte in the sample.

Vitamin D3 was analyzed as cholecalciferol at the Diagnostic Center for Population and Animal Health in 2010 (Lansing, Michigan USA) and Medallion Labs in 2021 (Minneapolis, WI). While the sample preparation methods remained the same between the two time points, the 2010 sample concentrations were determined by HPLC with a photodiode array detector and 2021

samples by LC-MS/MS. An internal standard was added to each sample and saponified in ethanoic KOH; it was then extracted into a heptane organic phaser and evaporated to dryness. The sample extract was then reconstituted, and fractions of the internal standard and D3 were collected via a solid phase extraction column. Both fractions were then concentrated and the concentration of D3 was determined using HPLC with photodiode array detector in 2010 or LC-MS/MS in 2021. Samples 3 and 8 unexpectedly exceeded Medallion Labs' testing limits, thereby requiring third party analysis, per standard procedures of the laboratory. Values were reported in µg/100 g and converted to IU/kg, to compare to label claims, and to µg/g, to compare 2010 and 2021 values.

Statistics

Descriptive statistics (median and range) were calculated with computer-based software.[m] When comparing concentrations of analytes, the following equation was used: (V2 – V1) / V1 X 100 = % Change. For assessments of label accuracy, V1 was the label claimed value and V2 was lab reported value. For comparisons between timepoints, V1 was the 2010 lab reported value and V2 was the 2021 lab reported value.

Results

FATTY ACIDS

All but one sample from 2010 had a label claim for minimum EPA and DHA content. Two samples did not meet the label claim for EPA and two did not meet the label claim for DHA; two samples met neither their EPA nor DHA label claim. Overall, median EPA concentration was 198.2 mg/mL (range 16.8 - 257.25 mg/mL), with variations compared to label claims of -34% to

+54%. Median DHA concentrations was 105.9 mg/ml (range 20.7 - 223.05 mg/mL), which varied from label claims by -43% to +78%. In 2021, all samples made minimum label claims for both EPA and DHA; one sample did not meet the label claim for DHA and one sample did not meet either EPA or DHA label claims. Median EPA concentration was 167.3 mg/ml (range 87.0 - 270.2 mg/mL) with variations compared to label claims of -48% to +151%. Median DHA concentration was 132.25 mg/ml (range 76.5 - 197.2 mg/mL) with variations compared to label claims of -58% to +132%.

Analyzed EPA concentrations between the between the timepoints changed, ranging from a 66% decrease to a 723% increase (Figure 1). Analyzed DHA concentrations between the timepoints also changed, ranging from a 55% decrease to a 392% increase (Figure 1).

MINERALS

For 2010, one product contained added zinc sulfate in its ingredient list, but made no minimum claim on its label. This sample contained the highest amount of zinc among 2010 samples (1400 ug/g), but was nearly undetectable in 2021. For 2021, one product contained added zinc gluconate and made a minimum claim (215 ug/g with no added zinc in 2010, 1.2 ug/g with added zinc in 2021), one product had a claim denying containing any detectable minerals (did contain 0.069 ug/g of selenium), and one product manufacturer provided an online source to verify the laboratory results of that specific product batch (which closely matched our analysis).

No sample from either time point contained any detectable concentrations of Mo, Cd, Hg, or Pb. Only 1 sample (from 2010) contained a detectable amount of Mn, while one sample from each timepoint had measurable concentrations of Cu (Table 1). Overall, As was detected in 3/22

samples (13.6%) and Se in 6/22 samples (27.3%). Fe was detected in all but one sample from 2010 but was not found in any sample from 2021 (overall 10/22 samples (45.5%)) (Table 1). Zn was the most prevalent and was detected overall in 14/22 samples (63.6%), including all samples from 2010 but only 2 from 2021 (Table 1).

When used at the suggested label dose, no products contained mineral concentrations that exceeded the PDE limits for human adults set by the USP.[12] For example, while 2 samples from each time point contained As, all concentrations were below the limit of 15 µg per day; the one sample with detectable As at both time points had different amounts (ranging from 2.0 to 2.35 µg/g in 2010, 0.8 to 3.1 µg/g in 2021).

PCB AROCHLORS

None of the samples from 2010 or 2021 had any detectable concentrations of PCB arochlors.

VITAMINS

While nine samples from 2010 had a detectable amount of vitamin A (median 0.59 IU/kg); range 0.447 – 322.1 IU/kg), only two products made a minimum content claim, and neither were met (41% to 77% below claim). Three samples from 2021 claimed minimum vitamin A concentrations, but only two of those contained detectable amounts; no sample met the label clam (13% to 38% below claim). Overall, five samples from 2021 contained a detectable amount of vitamin A (median 323.33 IU/kg; range 123.3 – 433.3 IU/kg). For the four products that had detectable concentrations of vitamin A at both time points, the 2021 values were higher in all cases, ranging from 35% to 52882% increase (Figure 2).

Vitamin A samples from 2010 ranged from 0.01% to 8.67% of the AAFCO minimum daily recommendation of 5000 IU and 0.04% to 25.52% of the NRC minimum recommended daily allowance of 1262.07 IU; 2021 samples ranged from 2.47% to 8.67% and 9.77% to 34.33% below respectively.

While 9/11 (81.8%) samples from 2010 had a detectable amount of vitamin D, only one product had a minimum content claim, and it was not met (62% below claim). Overall, median vitamin D content of samples from 2010 was 2.6 IU/kg (median 2.6 IU/kg, range 0.52 – 29.6). Similarly, only one product in 2021 had a claim for minimum vitamin D content, but was also not met (74% below claim). Overall, four samples from 2021 had detectable amounts of vitamin D (median 8.6 IU/kg, range 4.2 – 15.8).

Changes in vitamin D3 concentrations between time points ranged from an 86% decrease to 508% increase (Figure 3). Vitamin D samples from 2010 ranged from 0.1% to 5.92% of the AAFCO minimum daily recommendation of 500 IU and 0.39 % to 21.76% below the NRC minimum recommended daily allowance of 136 IU; 2021 samples ranged from 0.83 % to 3.16% below and 3.39% to 11.62% below respectively.

Discussion

Comparisons of the same products from two different time points showed evidence of supplement formulation changes over an eleven-year period. This may reflect differences in raw materials sourcing, processing, and/or quality control and purification procedures. As the majority of these supplements are made with fish oil, minerals that can be a cause for concern accumulate most in fish liver – a 2022 study from Turkey concluded that available fish oil

supplements for human consumption may still contain amounts of Pb that exceed the recommended daily limits.[14] Some analytes were never or rarely detected, and appear to be of little concern with regard to fish oil supplements for pets, such as PCBs, Mn, Cd, Hg, Pb, and As. Others such as iron and zinc appeared to change more globally, with both essential minerals being commonly detected in 2010 and then rarely or not detected in 2021. The cause of this in unclear, but seems unlikely to be related to analysis methodology given the same laboratory procedures and detection limited were used at both timepoints.

Regulation of pet supplement products may be variable. As of 2020, the FDA has withdrawn its classifications of supplements for companion animal use (Compliance Policy Guide Sec. 690.100), and historically held the position that the Dietary Supplement Health and Education Act (DSHEA) of 1994, under which human supplements are regulated, "should not apply to products intended for animals".[7,15,16] As such, nutritional supplements for animals are regulated under the category of animal feed, unless they are explicitly approved for use as drugs. In addition, the NRC has recommended that supplements intended for animal use should be manufactured such that the products adhere to the nutritional standards set by the AAFCO and the USP.[17] As such, ingredients included in dietary supplements must have a recognized ingredient definition, or be an approved food additive that is "Generally Recognized As Safe" (GRAS) under the FFDCA. The use of any other ingredient may be subject to state or FDA enforcement action.[17] To be considered GRAS, the ingredient must only be used for its intended purposes (for example, cinnamon can be included in a product only as a flavoring, and not with a claim to lower cholesterol). An ingredient can lose its GRAS status if its concentration in a product exceeds upper safe limits and poses a toxicity risk (such as excess cholecalciferol poses

severe adverse effects).[17] Manufacturers must report to FDA if an issue with a animal food (including supplements) would be expected to result in serious adverse health effects. In addition, organizations like the American Society for the Prevention of Cruelty to Animals (ASPCA) and the National Animal Supplement Council (NASC) have voluntary adverse event reporting systems, where registered manufacturers ("members") report any adverse effect of their products.[17]

The current AAFCO Dog Food Nutrient Profiles Based on Dry Matter includes a minimum requirement of crude fat for adult maintenance of 5.5% and for growth and reproduction of 8.5%. In addition, there are minimum values for alpha-linolenic acid (ALA) and EPA+DHA for canine growth and reproduction, and for linoleic acid (LA) for both life stage categories.[8] In addition, there is a maximum ratio applying to both life stage categories for specific omega-6 : omega-3 FAs (LA+arachidonic acid : EPA+DHA) of 30 : 1. The NRC guidelines also include ALA and EPA+DHA as essential for canine growth and reproduction, as well as for maintenance.[8,9] Since EPA and DHA are also used for therapeutic benefit, veterinarians often recommend supplementation for various conditions.[18] Recommended doses for disease management are often higher than the label guidelines. Although the current study did not find concerning concentrations of the selected essential nutrients and heavy metals, excessive intake may still occur if very high amounts are administered. Veterinarians should ensure that their recommended products are purified and contain little to none of any potentially toxic components (especially those with lower limits for safe intake such as vitamin D and selenium). In addition, we found many instances at both timepoints where products did not meet (and in some cases, did not closely approximate) claimed minimum concentrations for EPA, DHA,

vitamin A, or vitamin D. While products may have improved in meeting their claims for EPA and DHA content (7 vs. 3 instances of negative discrepancies in 2010 and 2021, respectively), claim accuracy for vitamin A and D concentrations remained very poor. Additionally, a claim was made by one product to be purified enough to contain no detectable mineral concentrations, yet when analyzed, did contain a detectable amount of selenium. Overall, there is room for much improvement with regard to minimum nutrient claims for fish oil labels.

In terms of assessing safety of both essential minerals and heavy metals in fish oil, little guidance is available for intake limits for dogs. While AAFCO has set a maximum concentration for commercial dog foods for selenium, this is a conservative value which was established in the absence of empirical data.[8] The USP sets widely recognized standards for the "quality, purity, strength, and consistency" of drugs and drug ingredients manufactured or sold for both humans and animals in the United States.[8,9] The standards set by the USP are legally recognized by the FDA and apply to medicine and food ingredients, as well as to human dietary supplements. Based on the PDE limits for adult humans set by USP, the current study did not find any samples above the threshold for any element. In addition, all samples evaluated in 2010 and only four samples evaluated in 2021 had one or more minerals detected at an amount above the minimum detectable limit. Regardless, contamination of fish oil products continues to be a concern, considering the adverse effects of heavy metal toxicity when consuming supplements. In general, the effects of toxicity of any of the evaluated minerals range from cancers, gastrointestinal distress, nervous system diseases, and mental and physical development delays. Certain minerals (including Mo and As) have an unclear role in companion animal nutrition[9], although in other species these are considered to be essential nutrients.

More research to define the role of these compounds for dogs is needed. In 2010, As was found to be detected in 2 of the 11 fish oil supplements samples tested and remained below the United States Pharmacopeia's (USP) oral limit for humans, ranging from 2.0 μg/g to 2.35 μg/g – in 2021, one sample's As concentration increased to 3.1 μg/g. We concluded that because the daily consumption of arsenic for a dog based on its body weight is relatively low, the risk of arsenic poisoning caused by these amounts would be unlikely.

The current study also assessed provision of vitamins A and D in fish oil supplements. The AAFCO Dog Food Nutrient Profiles for adult maintenance includes a wide limit range for vitamin A (5000 to 250,000 IU /kg dry matter).[8] In 2021, samples ranged from 2.47% to 9% of the minimum AAFCO recommendation for vitamin A, if consuming a fish oil supplement as the sole source. This is a slight increase compared to the 2010 samples, which ranged from 0.01% to 6.0% of the minimum AAFCO recommendations. Given the 50-fold range in safe intake, fish oil appears to be a minor contribution to the overall vitamin A intake, and toxicity concerns are unlikely even when used at higher doses to achieve therapeutic intakes of omega-3 FAs. Of course, 5/22 products made claims for minimum vitamin A concentrations, and no samples contained these minimum amounts. Depending on the form(s) of vitamin A present, degradation due to exposure to light and air could be one plausible reason for this finding. All but one sample contained some form of vitamin E (including "mixed tocopherols") on the ingredient list, and all assessed products were within their "use by" dates. Neither vitamin E content nor presence of FA oxidation products were assessed in this study; manufacturers should utilize multifaceted approached to maximize product stability. This may be another area

for targeted improvements in quality assurance procedures, in order to assure both regulatory officials and consumers of accurate labeling.

Compared to that for vitamin A, the AAFCO Dog Food Nutrient Profile for adult maintenance has a narrower range for vitamin D in commercial canine diets (500 to 3000 IU /kg dry matter).[8] While 13/22 samples had detectable vitamin D, the amount only ranged up to 5.9% of the minimum AAFCO recommendation for vitamin D, if consuming a fish oil supplement as the sole source. The vitamin D provided by supplementation does not consider the overall vitamin D consumption from other sources, including the main daily diet. While intake from different diets is expected to be variable, toxicity concerns are possible but unlikely. Similar to the case for vitamin A, label claims for vitamin D were also inaccurate. While only one sample at each timepoint made a claim for minimum vitamin D concentration (sample 2/7), both instances had analyzed amounts that fell significantly short of the claim. Manufacturers of fish oil products should re-evaluate and minimum content claims and ensure compliance.

LIMITATIONS AND CONCLUSIONS

The central objective of this study was to evaluate the composition, label claims, and general safety of fish oil supplement samples analyzed at two timepoints. Overall, few safety issues were identified, with no detectable concentrations of many contaminants of concern. Other analytes were present at levels assessed to be of low risk when used in recommended amounts. As such, a conclusion can be made in favor of manufacturers continuing to produce supplements from pure ingredients, with good processing techniques, with cleaner equipment, and/or with adequate quality control practices. Screening for contaminants continues to be

warranted. However, minimum content claims for omega-3 FAs and for vitamins A and D were often inaccurate. Formulation errors, poor quality control, and product consistency or stability issues may all have contributed to this finding. Improving consistency and ensuring adherence to label guarantees is an important goal for manufacturers. Given that all but one sample included vitamin E for stability, synthetic preservatives or the use of pre-sealing nitrogen flushing of containers may help maintain the nutritional integrity of fish oil products.

One potential improvement for better regulatory guidance would be the generation of empiric data to describe safe intakes of various essential minerals and heavy metals for dogs. Finally, improving monitoring and enforcement activities of state and federal authorities is important for protection of consumers and their pets. Label claims must be accurate, and noncompliance may be a widespread problem given the findings reported in this study.

Conflicts of Interest

JAL is an investigator in clinical trials and other research partly or fully sponsored by Royal Canin, Nature's Variety Instinct, and Nestle Purina PetCare. She develops educational materials for Brief Media, Mark Morris Institute, and HealthyPet magazine, and has served as consultant for Elanco Animal Health. JAL participates in continuing education events, as a speaker & as an attendee, sponsored/organized by Royal Canin, Nestle Purina PetCare, Nature's Variety Instinct, and Hill's Pet Nutrition.

AJF is the Scientific Director of the Amino Acid Laboratory and the Feline Research Laboratories at the University of California, Davis (UCD) that provides research support on a fee for service basis. This did not lead to a conflict of interest or influence collection or interpretation of results.

AJF advised Synergy Food Ingredients, Clorox, and received a grant from Nutro and remuneration for lectures, or as an advisor on behalf of Nestlé Purina PetCare, Mars Petcare, Hill's Pet Nutrition and the Pet Food and Mark Morris Institutes. A nutrition resident received funds from the Hill's Pet Nutrition Resident Clinical Study Grants program; AJF collaborated on the resulting research project. The Veterinary Medical Teaching Hospital at UCD receives partial support for a Nutrition Technician from Nestlé Purina PetCare and its veterinary nutrition program from Nestlé Purina, Mars Petcare and Hill's Pet Nutrition.

JAL and AJF are both faculty in the Nutrition Service of the Veterinary Medical Teaching Hospital at UCD. A nutrition resident received funds from Hill's Pet Nutrition Resident Clinical Study Grants program; AJF and A.J.L. collaborated on the research project. The Veterinary Medical Teaching Hospital receives partial support for a Nutrition Technician from Nestlé Purina PetCare and its veterinary nutrition program from Nestlé Purina, Mars Petcare, and Hill's Pet Nutrition.

Funding

LEH was supported by the Nestle Purina Student Fellowship at the time this study was conducted in 2010 and was a participant in the University of California, Davis School of Veterinary Medicine's Students Training in Advanced Research (STAR) Program. The study was also supported by the Sommer Endowment from the Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis.

Acknowledgments

LEH collected the samples and presented the results in poster abstract format as part of her STAR Program in 2010 at the University of California, Davis. LRO collected the samples and

presented the overall results in a thesis format as part of her Graduate Group in Nutritional Biology program in 2023 at University of California, Davis.

Author Contributions

All authors were involved in hypothesis generation and experimental design. LEH and AJF identified the supplements and organized the samples in 2010; LRO and AJF did the same in 2021. All authors were involved in interpreting and analyzing the results for both respective time points. LRO, JAL, and AJF were major contributors in writing the manuscript. All authors contributed, read, and approved the final manuscript.

Footnotes

[a] Welactin® Canine, Nutramax Laboratories Veterinary Sciences, Inc., Lancaster, SC, USA

[b] Aller-G, Vetoquinol, Lure, France

[c] Nutri-Vet Wild Alaskan Salmon Oil, Nutri-Vet, LLC, Boise, ID, USA

[d] USA Super Pure Omega-3, PetMed Express, Inc., Delray Beach, FL, USA

[e] EicosaDerm®, Dechra, Lostock Gralam, UK (2021 only)

[f] Canine Omega3®, Ascenta, Boynton Beach, FL, USA

[g] Dr. Harvey's Health & Shine Fish Oil, Dr. Harvey's, Atlantic Highlands, NJ, USA

[h] Well & Good Skin & Coat Omega-3, Petco Animal Supplies, Inc., San Diego, CA, USA

[i] Ultra EFA, Rx Vitamins, Elmsford, NY,

[j] Lipiderm®, International Veterinary Sciences, Orangeburg, NY, USA

[k] Only Natural Salmon Oil, Only Natural Pet, Boulder, CO, USA

[l] Grizzly Salmon Oil, Grizzly Pet Products, Woodinville, WA, USA (2010 only – discontinued)

[m] Microsoft Office Excel 2022, Microsoft Corp, Redmond, Wash.

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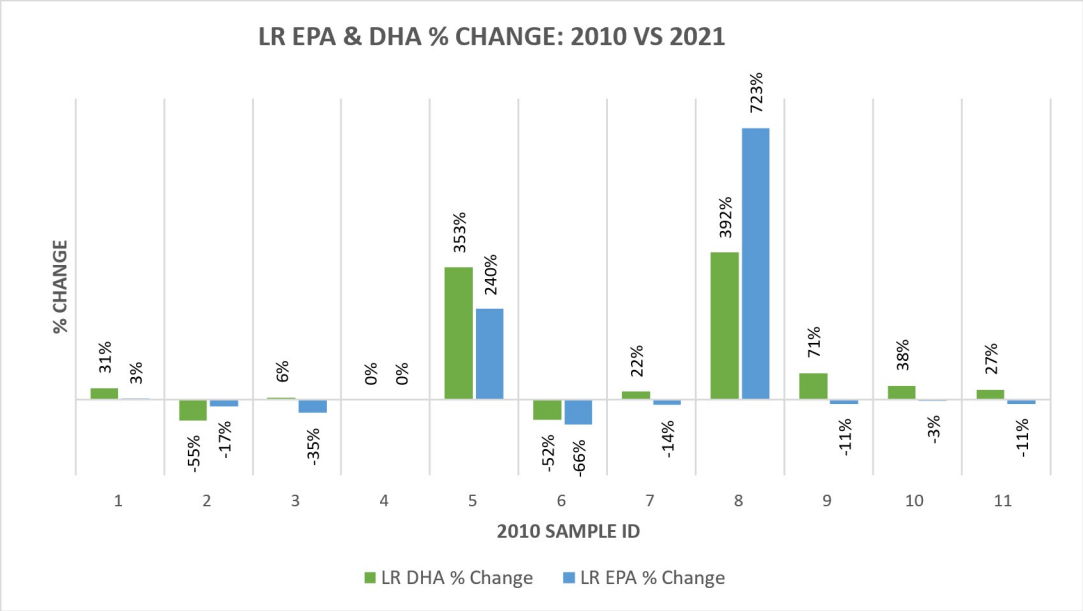

Figure 1. Changes in analyzed EPA and DHA concentrations in samples of fish oil supplements for pets Lab Result (LR) EPA and DHA Percent Change: 2010 vs 2021

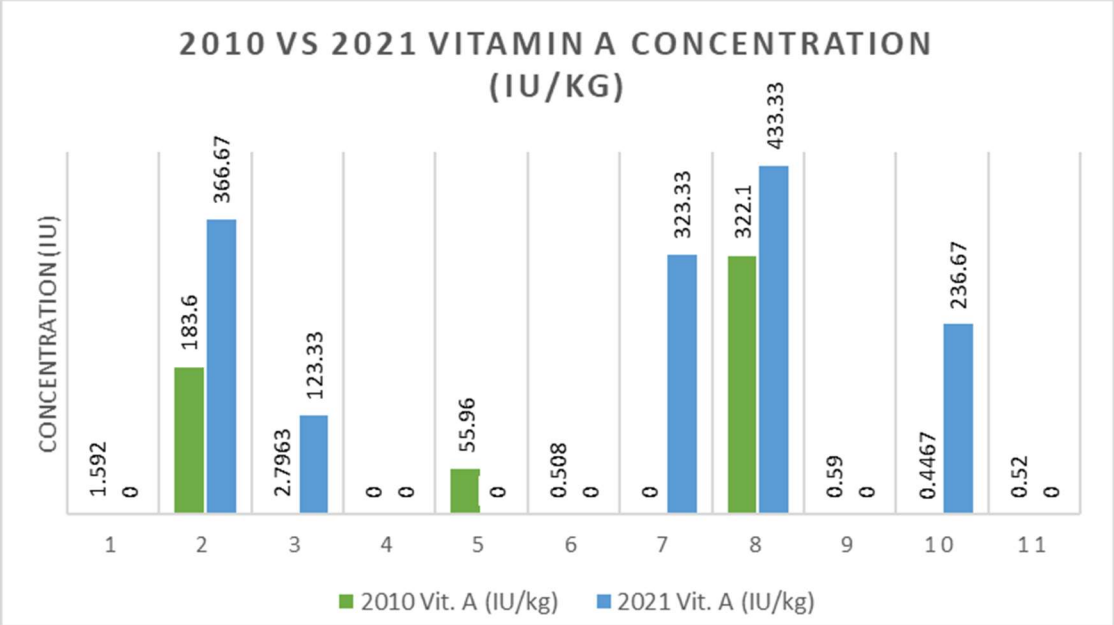

Figure 2. Changes in analyzed vitamin A concentrations in samples of fish oil supplements for pets

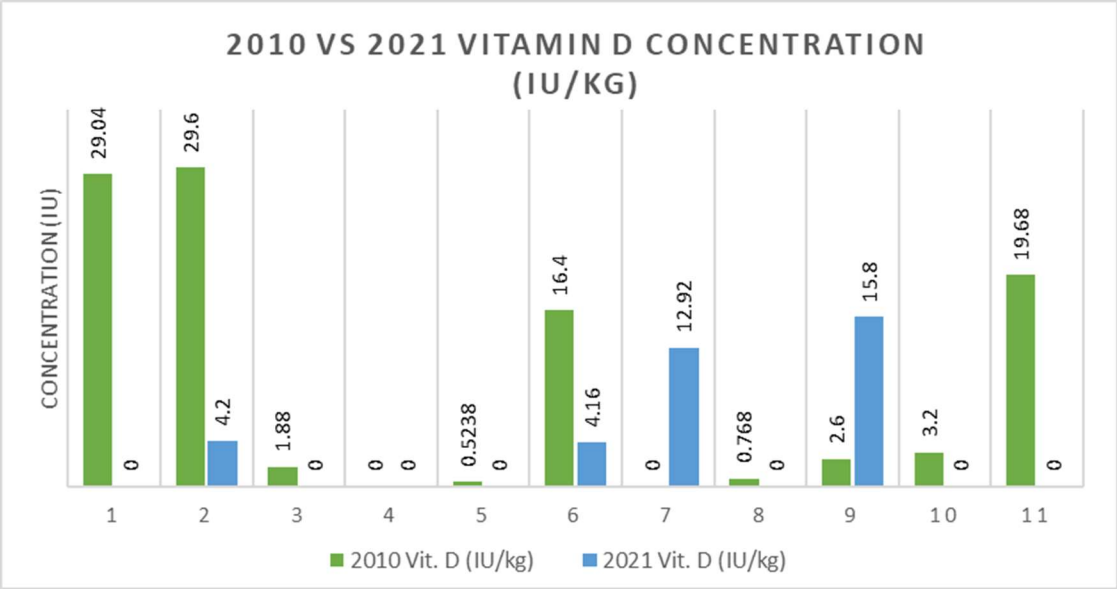

Figure 3. Changes in analyzed vitamin D (cholecalciferol) concentrations in samples of fish oil supplements for pets

Table 1. Eleven-year interval analysis of mineral concentrations in samples of fish oil supplements marketed for pets*

2010 / 2021	Manganese (ug/g)		Iron (ug/g)		Copper (ug/g)		Zinc (ug/g)		Arsenic (ug/g)		Selenium (ug/g)	
1 / 1	N/D	N/D	0.805	N/D	N/D	N/D	1.02	N/D	N/D	N/D	0.0345	N/D
2 / 7	N/D	N/D	0.66	N/D	7.4	N/D	1.45	N/D	N/D	N/D	N/D	N/D
3 / 6	N/D	N/D	0.45	N/D	N/D	N/D	2.7	N/D	N/D	N/D	N/D	0.069
4 / 5*	N/D	N/D	1.14	N/D	N/D	N/D	1.1	N/D	2	N/D	0.0565	N/D
5 / 4	1.35	N/D	4.45	N/D	N/D	N/D	215	1.2	N/D	N/D	N/D	N/D
6 / 2	N/D	N/D	0.605	N/D	N/D	0.09	2.15	N/D	N/D	0.8	N/D	0.073
7 / 3	N/D	N/D	0.665	N/D	N/D	N/D	4.26	0.52	2.35	3.1	0.021	0.11
8 / 10	N/D	N/D	0.365	N/D	N/D	N/D	1400	N/D	N/D	N/D	N/D	N/D
9 / 8	N/D	N/D	0.43	N/D	N/D	N/D	0.79	N/D	N/D	N/D	N/D	N/D
10 / 9	N/D	N/D	0.34	N/D	N/D	N/D	0.745	N/D	N/D	N/D	0.0255	N/D
11 / 11	N/D	N/D	N/D	N/D	N/D	N/D	0.23	N/D	N/D	N/D	N/D	N/D
Median	1.35		0.6325		3.745		1.2		2.175		0.0565	
Range	0 - 1.35		0.34 - 4.45		0.09 - 7.4		0.23 - 1400		0.8 - 3.1		0.0255 - 0.11	

*2010 Samples in grey columns; 2021 Samples in white columns, N/D = Not Detectable

Molybdenum, cadmium, mercury, and lead were not detected in any samples (detection limit: 0.050 ug/g).

Detection limits: 0.050 ug/g for manganese, iron, arsenic, and selenium; 0.070 ug/g for copper, and 0.080 ug/g for zinc.