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Lipid source and kibble matrix determine oxidative stability and n-3 fatty acid loss in coated extruded dog food

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Abstract

Lipid oxidation is a key determinant of nutritional quality, palatability, and shelf life in extruded dog foods. This study evaluated how lipid source, kibble matrix, and antioxidant inclusion influence oxidation and shelf life of coated extruded dog kibble. Two kibble matrices (grain-inclusive and grain-free) were formulated to meet adult maintenance recommendations and externally coated at 10% (as-is) with 1 of 10 lipid sources [algae, fish, flax, camelina, canola, sunflower, coconut, black soldier fly larvae oil, chicken fat, duck fat] or left uncoated (control). Each coated treatment was produced with or without a natural antioxidant blend (tocopherols + rosemary extract; 200 ppm), then stored under ambient (22°C, 50% RH; 15 mo) and accelerated conditions (37°C, 40% RH; 3 mo). Oxidation was assessed via peroxide value (PV), hexanal, 2,4-decadienal, and tocopherols, and time to failure was defined as PV $\geq$ 5 mEq/kg or (hexanal + 2,4-decadienal) $\geq$ 25 ppm. Proximate, mineral, and fatty acid profiles were assessed during ambient storage. Under ambient storage, PV and aldehydes increased with time ($P < 0.05$), and oil source influenced oxidation markers and fatty acid losses ($P \leq 0.01$). Oils rich in polyunsaturated fatty acids (PUFA; algae, flax, and camelina) had greater oxidation, more rapid tocopherol depletion, and shorter time to failure than more saturated lipid sources (coconut and poultry fats). Baseline double bond index (DBI) predicted oxidative failure ($P < 0.0001$). Mean eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations across treatments decreased by 98.7% and 77.9%, respectively, from 0 to 15 mo. Grain-inclusive kibble was associated with greater aldehyde accumulation ($P < 0.05$), greater antioxidant depletion ($P < 0.0001$), and shorter time to failure than grain-free kibble ($P = 0.001$). Antioxidant inclusion increased baseline tocopherol concentrations but did not consistently affect PV or secondary aldehyde concentrations or extend time to failure across oil types at the inclusion level used ($P > 0.10$). In this article, lipid source was the dominant determinant of oxidative stability, with baseline DBI supporting degree of fatty acid unsaturation as an important contributor, alongside meaningful contributions from the kibble matrix. Effective preservation of long-chain n-3 PUFA requires formulation-specific stabilization strategies, beyond a uniform antioxidant solution, or alternative delivery approaches.

Lay Summary

Fats and oils are added to dog foods to improve nutrition, taste, and energy content, but they can spoil over time through oxidation. Oxidation reduces food quality, causes off-odors, and can lead to nutrient loss. This article examined how different oil types, kibble formulations, and added antioxidants influence fat stability and shelf life in dry dog food. Two types of kibble (grain-inclusive and grain-free) were coated with a variety of oils/fats and stored under long-term normal (15 months) and short-term warm (3 months) conditions. Oils rich in highly unsaturated fats (algae, flax, and camelina) spoiled faster than more saturated fats (coconut oil and poultry fats). The omega-3 fatty acids found in marine oils (eicosapentaenoic acid and docosahexaenoic acid) declined during storage. Kibble type also mattered, where grain-inclusive kibble had a shorter average shelf life than grain-free kibble. Using a single natural antioxidant at a single dose did not consistently prevent spoilage across all oil/fat types, confirming the need to closely evaluate the targeted raw material being used and match its needs with the appropriate antioxidant and dosage rate. Overall, oil choice plays the biggest role in shelf life, and preserving marine-derived omega-3 requires more targeted stabilization strategies.

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Keywords docosahexaenoic acid, eicosapentaenoic acid, kibble, lipid oxidation, polyunsaturated fatty acids, shelf life

Abbreviations: AA, arachidonic acid; AAFCO, Association of American Feed Control Officials; ALA, α-linolenic acid; AOAC, Association of Official Analytical Chemists; AOCS, American Oil Chemists' Society; BSFL, black soldier fly larvae; DBI, double bond index; DHA, docosahexaenoic acid; DMB, dry matter basis; EPA, eicosapentaenoic acid; FOX II, ferrous oxidation–xylenol orange II assay; GC-FID, gas chromatography–flame ionization detection; HPLC-FLD, high-performance liquid chromatography–fluorescence detection; ISO, International Organization for Standardization; LA, linoleic acid; MUFA, monounsaturated fatty acids; PET, polyethylene terephthalate; PUFA, polyunsaturated fatty acids; PV, peroxide value; SFA, saturated fatty acids

Introduction

Extruded dry kibble is the most fed commercial pet food format worldwide. Recent surveys estimate that approximately 80% of dogs in the United States receive dry food as the main part of their diet (O'Brien et al. 2024). Dry pet foods are favored by consumers due to convenience, affordability, and extended shelf life, which is typically labeled as 12–15 months under ambient storage conditions (Chanadang et al. 2016; Vendramini et al. 2020). However, the ability of an extruded diet to maintain nutritional quality and palatability across this declared shelf life depends largely on lipid stability and oxidation during storage (Gross et al. 1994; Lin et al. 1998; Jacobsen 1999; Chanadang et al. 2016; Hołda and Głogowski 2016; Beer et al. 2023).

Dietary fats play important roles in pet foods, including the provision of fatty acids, contribution to energy density, and enhancement of palatability. Although adequate intake and recommended allowance values have been published for several fatty acids in adult dogs (National Research Council 2006), concentrations of individual fatty acids in commercial dog foods vary with life stage, ingredient composition, total dietary fat, and intended nutritional targets or functional claims. At the same time, lipids can be among the most chemically unstable components of extruded diets. Lipid oxidation proceeds through free-radical chain reactions, resulting in the formation of primary oxidation products such as hydroperoxides, followed by secondary products including aldehydes that are associated with rancid odor and flavor (Jacobsen 1999). In dry pet foods, these oxidative reactions are of greater concern than microbial spoilage, as extrusion and drying typically reduce water activity to levels that inhibit microbial growth (Park et al. 2025). In addition, the formation of these primary and secondary oxidative products can negatively influence palatability and diet acceptance by the pet, while also adversely affecting sensory characteristics important to the pet owner (Lin et al. 1998; Jacobsen 1999; Chanadang et al. 2016; Beer et al. 2023), and can additionally have negative consequences on animal health, particularly for young growing animals and those that are immunocompromised (Turek et al. 2003).

However, the susceptibility of a dry pet food to lipid oxidation is influenced not only by its fatty acid composition but can also be influenced by the physical and chemical properties of the kibble matrix. Fats are commonly applied post-extrusion as surface coatings (Kim et al. 2025), placing them directly at the air–solid interface where they are exposed to oxygen and pro-oxidant components of the kibble matrix. In particular, the concentrations of metals, like copper and iron, are of importance when considering oxidative stability and shelf life (Dainton et al. 2021). In addition, properties such as extrudate expansion and internal structure affect oxidation rates, with highly expanded kibbles exhibiting greater susceptibility to oxidative deterioration due to increased surface area and oxygen accessibility (Lin et al. 1998); highlighting that lipid oxidation in finished pet foods cannot be predicted from bulk oil composition alone. Differences in kibble matrix composition are particularly relevant when comparing grain-inclusive and grain-free formulations. Grain-free diets, which often rely on pulses and tubers as primary carbohydrate sources, require greater moisture input and specific mechanical energy during extrusion to achieve comparable bulk density; leading to differences in kibble structure and moisture dynamics (Pezzali and Aldrich 2019). Studies evaluating pulse-rich and grain-free diets have found that greater water-holding capacity and altered drying behavior can increase the risk of quality deterioration during storage (Crosbie et al. 2024).

In parallel, the pet food industry is increasingly incorporating alternative and more sustainable lipid sources into dry diets. These include long-chain n-3–rich algae oils, high-α-linolenic acid plant oils, and insect-derived fats such as black soldier fly larvae (BSFL) oil. While these ingredients offer potential environmental and nutritional benefits, they differ substantially in fatty acid composition and intrinsic oxidative stability. Long-chain n-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are particularly susceptible to degradation during storage of extruded pet foods, with up to 33% losses after 12 months of storage (Kara 2021). In contrast, insect-derived fats are characterized by greater proportions of saturated (SFA) and monounsaturated fatty acids (MUFA; Jayanegara et al. 2020; Li et al. 2022), which may offer greater oxidative stability. However, limited data exist comparing the behavior of these diverse lipid sources within finished extruded kibble matrices.

Despite the importance of lipid stability for nutritional quality and palatability, few studies have systematically evaluated the combined effects of lipid source, kibble matrix, and antioxidant inclusion on oxidation and shelf life in extruded pet foods over commercially relevant storage periods. Therefore, the objective of the present study was to evaluate the oxidative stability of extruded dog kibble coated with a range of lipid sources differing in fatty acid composition, including conventional animal fats, marine oils, plant oils, coconut and insect-derived oil. Two kibble matrices (grain-inclusive and grain-free) were produced and coated with each lipid source, with or without a natural antioxidant blend, and stored under long-term ambient and accelerated conditions. Oxidative stability was assessed using several complementary indicators, including peroxide value (PV), secondary aldehyde formation, tocopherol depletion, and changes in fatty acid composition. This design enabled assessment of

how formulation and storage conditions jointly influence oxidative stability in commercially relevant extruded dog foods.

Materials and methods

Experimental diets and treatments

Two experimental diets were formulated to meet or exceed the nutrient recommendations for adult maintenance dogs according to the Association of American Feed Control Officials (AAFCO 2025). Both diets were chicken-based and formulated to achieve similar macronutrient profiles, including target concentrations of crude protein, crude fat, and crude fiber. The first formulation included grains (millet, oats, and sorghum) while the second included pulses (chickpeas, lentils, and peas). Both diets were produced at the Agri-Food Discovery Place at the University of Alberta (Edmonton, AB, Canada), using a Wenger X-115 single-screw extruder (Wenger Corporation, Owatonna, MN, USA), and produced to target a water activity of ≤ 0.67. After extrusion and drying, kibble was left to cool under ambient conditions. Within 48 h, diets were shipped to Champion Petfoods' Research and Innovation Centre (Morinville, AB, Canada), where external coatings were applied. Each diet was coated with one of ten fats or oils: fish oil (pollock), algae oil, flaxseed oil, camelina oil, coconut oil, BSFL oil, chicken fat, duck fat, sunflower oil, or canola oil. Lipid sources were selected to reflect a broad range of fatty acid profiles, ingredient novelty, and current or emerging relevance to the pet food industry. Where possible, oils and fats that had not been pre-treated with antioxidants were purchased, however, this was not possible in all cases. Available supplier information indicated that the flax, camelina, coconut, and BSFL oils contained no added antioxidants; the algae and fish oils contained added natural tocopherols, although the concentration was not reported; canola oil, chicken fat, and duck fat contained natural tocopherols at approximately 500–1,000 ppm; and sunflower oil contained a natural antioxidant at approximately 1,000–1,500 ppm. Detailed product-specific fatty acid profiles of the raw lipid sources were not available; however, fatty acid composition of the finished coated treatments was quantified at baseline as described below. Upon arrival to Champion Petfoods, all oils were tested for PV using a commercially available ferric ion–based colorimetric lipid hydroperoxide assay (Lipid Peroxide, SafTest Test Kit, MP Biomedicals, Irvine, CA, USA), to ensure that they were not oxidized upon arrival and before coating, with acceptance defined as PV $\leq$ 5 mEq/kg. Oils and fats were manually coated onto both kibble types at 10% (as-is basis) of total kibble weight (1.5 kg oil or fat/15 kg diet) using a rotating coating drum. Coconut oil and BSFL oil were heated to 25°C, to melt them for application. Each kibble base also included an uncoated control, where no oil or fat was coated. All experimental oils and fats were applied exclusively as post-extrusion surface coatings and were not incorporated into the kibble matrix before or during extrusion.

For each of the 10 oil/fat treatments, two antioxidant conditions were applied: with or without a natural antioxidant consisting of mixed tocopherols and rosemary extract (NATUROX R30 Liquid, Kemin Nutrisurance Inc, Des Moines, IA, USA). A natural antioxidant system was selected to reflect its commercial relevance to the pet food industry. When included, the antioxidant was mixed into the oil/fat pre-coating and top-coated with the oil/fat onto the kibble surface at an inclusion of 200 ppm antioxidant/kg diet. The inclusion level was selected in consultation with the manufacturer (Kemin Nutrisurance, Inc) as the recommended application rate for the 10% external lipid coating used in this article. The uncoated control was not treated with antioxidant. No antioxidants were added internally to the kibble matrix during formulation. This factorial design resulted in a total of 42 unique treatments [2 kibble bases $\times$ (10 oils + 1 control) $\times$ 2 antioxidant treatments] (Table 1). Once coated, 5 kg of each treatment (1 kg $\times$ 5) was weighed out and transferred to metallized polyethylene (PET) stand-up pouches with zipper seals.

Shelf life and oxidative measures

Within two weeks of coating, one PET pouch (1 kg total) of each treatment was shipped from Champion Petfoods Research and Innovation Centre to Kemin Nutrisurance Customer Lab Services (Des Moines, IA, USA). At the same time, two PET pouches of each treatment were shipped to the Department of Animal Biosciences at the University of Guelph (Guelph, ON, Canada). Shipments arrived within 1–2 days at both destinations, and upon arrival, the treatments were transferred from the PET pouches to plastic sandwich bags. Kibble was kept stored at ambient storage conditions (22°C and 50% relative humidity) for 15 months in the Kemin Nutrisurance analytical lab (Des Moines, IA, USA) and at accelerated storage conditions (37 °C and 40% relative humidity) for 3 months at the Guelph Food Innovation Centre, University of Guelph (Guelph, ON, Canada). The remaining PET treatment pouches remained stored at Champion Petfoods at ambient storage conditions (22°C).

Samples from treatments stored at accelerated conditions at the University of Guelph were shipped monthly to the Kemin Nutrisurance analytical lab (Kemin Nutrisurance Inc, Des Moines, IA, USA). Treatments stored at ambient conditions were tested every 3 months for 15 months, while the treatments stored at accelerated conditions were tested monthly in singlet for 3 months for the following: PV, hexanal, 2, 4-decadienal (decadienal) and tocopherols [total tocopherols and tocopherol isomers: alpha (α), gamma (γ), and delta (δ)]. Kibble samples were ground before analysis, and analytes of interest were extracted using appropriate organic solvents. Peroxide value was determined using a ferrous oxidation–xylenol orange II assay with UV–visible spectrophotometric detection, consistent with established American Oil Chemist Society (AOCS 1994) reference methods (Cd 8 b-90). Secondary lipid oxidation products, including hexanal and 2,4-decadienal, were quantified by gas chromatography with flame ionization detection (GC-FID). Total tocopherols and individual tocopherol isomers (α, γ, and δ) were quantified by high-performance liquid chromatography with fluorescence detection. The upper and lower limit of detection for PV were 7.2 and 0.1 mEq/kg, respectively. The lower limits of detection for hexanal, decadienal, and tocopherols were 2,1 and 10 ppm, respectively. During each testing interval, the Ziploc bags were opened and a small sample was taken for testing (100 g) from each bag, before resealing and returning to storage.

Table 1 Treatment combinations used to evaluate the effects of kibble matrix [grain-inclusive[a](grain) or grain-free[b] (GF)], oil/fat coating, and antioxidant inclusion on oxidative stability of extruded dog kibble during 15 mo ambient and 3 mo accelerated storage conditions.

Treatment ID	Kibble type	Oil/fat coating[c]	Antioxidant inclusion[d]
1	Grain	Fish	+
2	Grain	Fish	–
3	Grain	Algae	+
4	Grain	Algae	–
5	Grain	Flax	+
6	Grain	Flax	–
7	Grain	Camelina	+
8	Grain	Camelina	–
9	Grain	Coconut	+
10	Grain	Coconut	–
11	Grain	BSFL	+
12	Grain	BSFL	–
13	Grain	Chicken	+
14	Grain	Chicken	–
15	Grain	Duck	+
16	Grain	Duck	–
17	Grain	Sunflower	+
18	Grain	Sunflower	–
19	Grain	Canola	+
20	Grain	Canola	–
21	Grain	Control	–
22	GF	Fish	+
23	GF	Fish	–
24	GF	Algae	+
25	GF	Algae	–
26	GF	Flax	+
27	GF	Flax	–
28	GF	Camelina	+
29	GF	Camelina	–
30	GF	Coconut	+
31	GF	Coconut	–
32	GF	BSFL	+
33	GF	BSFL	–
34	GF	Chicken	+
35	GF	Chicken	–
36	GF	Duck	+
37	GF	Duck	–
38	GF	Sunflower	+
39	GF	Sunflower	–
40	GF	Canola	+
41	GF	Canola	–
42	GF	Control	–

[a]Grain-inclusive kibble: chicken meal, oat groats, sorghum, millet, turkey meal, chicken, chicken and turkey giblets, miscanthus grass, egg, potassium chloride, salt, vitamin B premix, choline chloride, vitamin ADE, zinc, kelp, vitamin E, taurine, copper.
[b]Grain-free kibble: chicken meal, pea starch, green and yellow peas, green and red lentils, chickpeas, chicken, chicken & turkey giblets, miscanthus grass, egg, potassium chloride, salt, vitamin B premix, choline chloride, vitamin ADE, zinc, kelp, vitamin E, taurine, copper.
[c]Oils and fats were applied as an external coating onto the kibble at 10%.
[d]NATUROX R30 Liquid (mixed tocopherols + rosemary extract), Kemin Nutrisurance Inc, Des Moines, IA, USA.

Proximate and mineral analysis

The proximate composition of the 42 treatments was analyzed in singlet at Champion Petfoods' Research and Innovation Centre (Morinville, AB, Canada), at 0, 6, and 15 mo, using methods described by the Association of Official Analytical Chemists (AOAC; Horwitz et al. 1970), AOCS (1994), and the International Organization for Standardization (ISO). This included the measurement of moisture (ISO 6496:1999; ISO 1999), crude protein (AOAC 992.15), crude fat (ISO 11085:2015; ISO 2015), ash (AOAC 942.05), and minerals (AOAC 985.01/2011.14 mod), including calcium, phosphorus, potassium, sodium, magnesium, iron, copper, manganese, and zinc.

Fatty acid analysis

A sample of each treatment was taken at baseline for fatty acid analysis in singlet. Baseline fatty acid analysis was done on a gas chromatograph (Agilent 6890 N gas chromatograph, Agilent Technologies, Santa Clara, CA, USA) with flame ionization detector (GC-FID; AOAC 996.06; Mérieux Biosciences, Burnaby, BC, Canada). After 15 months of ambient storage at Champion Pet Foods, samples were re-analyzed for fatty acid profile using the same methodology (AOAC 996.06) at Champion Petfoods' Research and Innovation Centre (Morinville, AB, Canada).

Statistical analysis

Data were analyzed using SAS (SAS Studio 3.82, SAS Institute Inc., Cary, NC, USA). Studentized residuals for all response variables were assessed for normality using the Shapiro–Wilk test and visual inspection of residual and scatter plots. Homogeneity of variance was evaluated by visual inspection of residual versus predicted value plots. For samples at ambient storage, oxidation markers (PV, hexanal, decadienal, and tocopherols) exhibited right-skewed distributions typical of chemical oxidation data; therefore, log transformations were applied before analysis to improve residual normality. Repeated measures analyses were conducted using the GLIMMIX procedure. For oxidation outcomes under ambient storage conditions (0, 3, 6, 9, 12, and 15 mo), two model structures were evaluated: (1) an uncoated versus coated model including time, kibble type, coating status, and the interactions time × kibble and time × coating status as fixed effects; and (2) a coated-only factorial model including time, kibble type, oil, antioxidant inclusion (antioxidant), and the interactions time × kibble, time × oil, time × antioxidant, and oil × antioxidant as fixed effects. For all repeated measures models, each kibble × oil × antioxidant combination was treated as the subject, and an autoregressive covariance structure [AR(1)] was specified for within-subject correlation; denominator degrees of freedom were estimated using the Kenward–Roger method.

To characterize oxidation rates under ambient storage conditions, per-sample slopes were estimated for each oxidation marker. For each experimental unit, linear regression was used to estimate the slope of log-transformed peroxide value, hexanal, decadienal, and antioxidant concentration as a function of time (0, 3, 6, 9, 12, and 15 mo), using PROC REG. Slope estimates were subsequently analyzed using PROC GLIMMIX. Two model

structures were evaluated: (1) a coated versus uncoated model including kibble type, coating status, and their interaction as fixed effects; and (2) a coated-only factorial model including kibble type, oil source, antioxidant inclusion, and the oil- × antioxidant interaction as fixed effects. Denominator degrees of freedom were estimated using the Kenward–Roger method.

For both ambient and accelerated storage, time to failure was calculated for each sample as the earliest time point at which the failure definition was met: PV ≥ 5 mEq/kg sample or secondary aldehydes (hexanal + decadienal) ≥ 25 ppm. Inferential analysis of time to failure was conducted using Cox proportional hazards regression (PROC PHREG), with treatment effects evaluated using Wald chi-square tests from joint (Type III) tests. Models were fit for only coated treatments to assess the effects of kibble type, oil source, antioxidant inclusion, and the oil- × antioxidant interaction. An additional model including both coated and uncoated treatments was used to evaluate coating status, kibble type, and their interaction.

Nutrient composition (moisture, protein, fat, ash, and minerals) analyzed under ambient storage conditions (0, 6, and 15 mo) was similarly analyzed using PROC GLIMMIX with time, kibble type, coating status, and the interactions time × kibble and time × coating status as fixed effects, with each kibble × oil × antioxidant treatment unit as the subject and an AR (1) covariance structure. Apart from moisture, nutrient composition was assessed and reported on a dry matter basis (DMB).

Fatty acid composition was analyzed under ambient storage using data collected at baseline (0 months) and end of storage (15 months). An intermediate time point (6 months) was excluded from statistical analysis due to anomalous increases in several fatty acids that were inconsistent with expected storage-related behavior and the fatty acid profiles of the oils and fats selected. Individual fatty acids were grouped into biologically relevant classes before analysis: saturated fatty acids (SFA; C12:0, C14:0, C16:0, C18:0), monounsaturated fatty acids (MUFA; C16:1, C18:1, C20:1, C22:1), n-3 polyunsaturated fatty acids [PUFA; α-linolenic acid (ALA), EPA, and DHA], and n-6 PUFA [linoleic acid (LA), arachidonic acid (AA), C18:2 trans, C18:3γ, C20:2, and C20:3 isomers]. Total PUFA was calculated as the sum of n-3 and n-6 PUFA. In addition, AA, LA, ALA, EPA, and DHA were analyzed individually. Fatty acid outcomes were log-transformed before analysis to address right-skewness and meet model assumptions. For each fatty acid class and individual fatty acids, a linear mixed-effects model was run using PROC GLIMMIX with the fixed effects of time, oil type, kibble type, antioxidant inclusion, and relevant two-way interactions (time × oil, time × kibble, time × antioxidant, and oil × antioxidant). Treatment unit (kibble × oil × antioxidant combination) was included as a random effect, with time treated as a repeated measure using a compound symmetry covariance structure to account for correlation between repeated measurements on the same sample. Degrees of freedom were estimated using the Kenward–Roger method.

To further evaluate the relationship between fatty acid composition and oxidative stability, a double bond index (DBI) was calculated for each coated treatment using its baseline fatty acid profile. Individual fatty acid concentrations were normalized to the total measured fatty acid concentration, and DBI was calculated as the sum of the relative proportion of each fatty acid multiplied by its number of double bonds. Baseline DBI was then evaluated as a continuous predictor of ambient time to oxidative failure using Cox proportional hazards regression (PROC PHREG). Kibble type and antioxidant inclusion were included as covariates to account for their potential effects on time to failure while evaluating the association between DBI and oxidative stability. Hazard ratios for DBI were expressed per 0.1-unit increase. The proportional hazards assumption was assessed using the Schoenfeld residual-based test.

Statistical significance was declared at $P \leq 0.05$, and trends were discussed when $0.05 < P \leq 0.10$. When significance occurred, a Tukey's post hoc test was performed for multiple comparisons. Log-transformed data were back-transformed to obtain the least square means (LSM). Results are expressed as LSM ± SEM.

Results

Oxidative markers and shelf life

Ambient storage (15 mo)

Ambient shelf life outcomes for PV, hexanal, decadienal, and tocopherol concentrations are presented in Figures 1–4.

Peroxide value

Under ambient storage conditions (15 mo), PV increased over time ($P_{Time} < 0.0001$). Coating status affected PV ($P_{Coated} = 0.027$), with uncoated control samples having a lower mean PV compared with coated treatments across storage. A tendency for a time × coating interaction was observed ($P_{Time \times Coated} = 0.065$), where PV increased more throughout the storage period in the coated treatments, as compared to the uncoated control samples. When accounting for both coated and uncoated samples, kibble type did not affect PV ($P_{Kibble} = 0.227$), and no time × kibble interaction was detected ($P_{Time \times Kibble} = 0.244$).

Within coated treatments, there was an effect of oil type on PV concentrations ($P_{Oil} < 0.0001$). Algae, flax, and camelina oils had the three greatest mean PV concentrations, with all three being greater than canola, sunflower, chicken, duck, BSFL, and coconut. Algae was additionally greater than fish and camelina and tended to be greater than flax. Coconut oil had the lowest mean PV concentration of all oil types analyzed. A time × oil interaction was detected ($P_{Time \times Oil} < 0.0001$), where algae oil had the greatest PV compared to all oils at the start of shelf life (0–3), but started to decline after 6 mo. The PV of flax, camelina and fish experienced a sharp increase at 6 mo. In comparison coconut, BSFL, duck and chicken remained steady throughout the 15 mo storage period, with coconut consistently being the lowest across all time points.

A tendency for a time × antioxidant interaction was observed ($P_{Time \times Antioxidant} = 0.091$). PV was comparable between antioxidant treatments at baseline. Over storage, PV increased in both groups, with larger numerical increases observed in antioxidant-treated samples from 6 to 15 mo. Within coated treatments, grain-inclusive kibble had greater mean PV values than grain-free kibble ($P_{Kibble} = 0.003$). Additionally, a time × kibble interaction was detected ($P_{Time \times Kibble} = 0.0422$), where grain-inclusive kibble had greater PV than grain-free kibble at 0–9 mo. However,

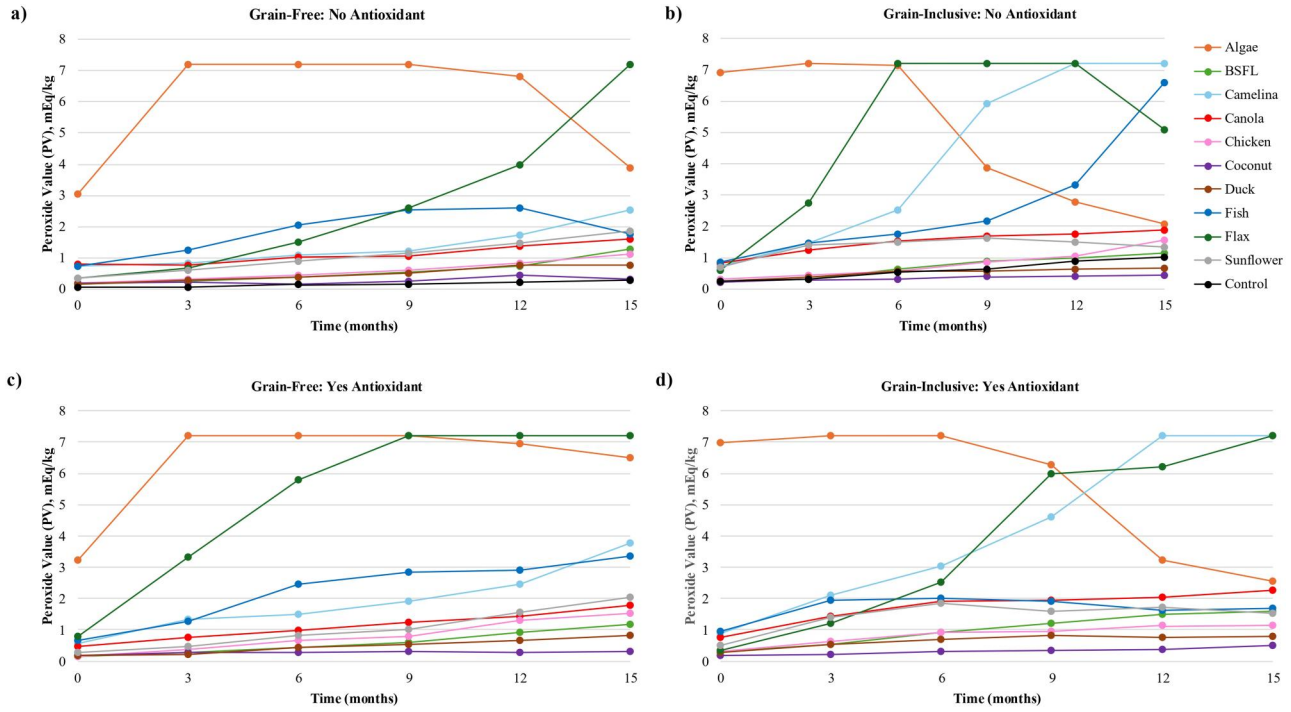


Figure 1 Peroxide value (PV; mEq/kg) of extruded dog kibble coated with different lipid sources during 15 mo of ambient storage (22°C, 50% RH). Panels represent: (a) grain-free kibble without antioxidant inclusion, (b) grain-inclusive kibble without antioxidant inclusion, (c) grain-free kibble with antioxidant inclusion, and (d) grain-inclusive kibble with antioxidant inclusion.

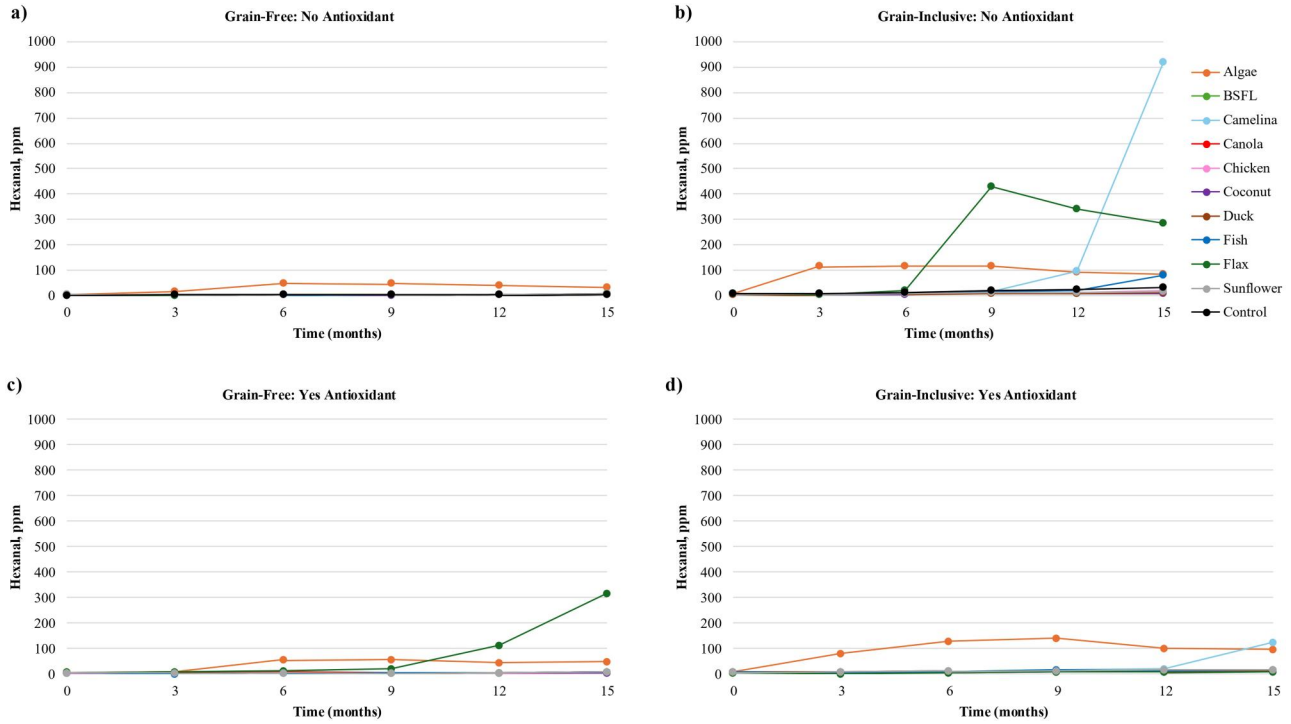


Figure 2 Hexanal concentrations (ppm) of extruded dog kibble coated with different lipid sources during 15 mo of ambient storage (22°C, 50% RH). Panels represent: (a) grain-free kibble without antioxidant inclusion, (b) grain-inclusive kibble without antioxidant inclusion, (c) grain-free kibble with antioxidant inclusion, and (d) grain-inclusive kibble with antioxidant inclusion.

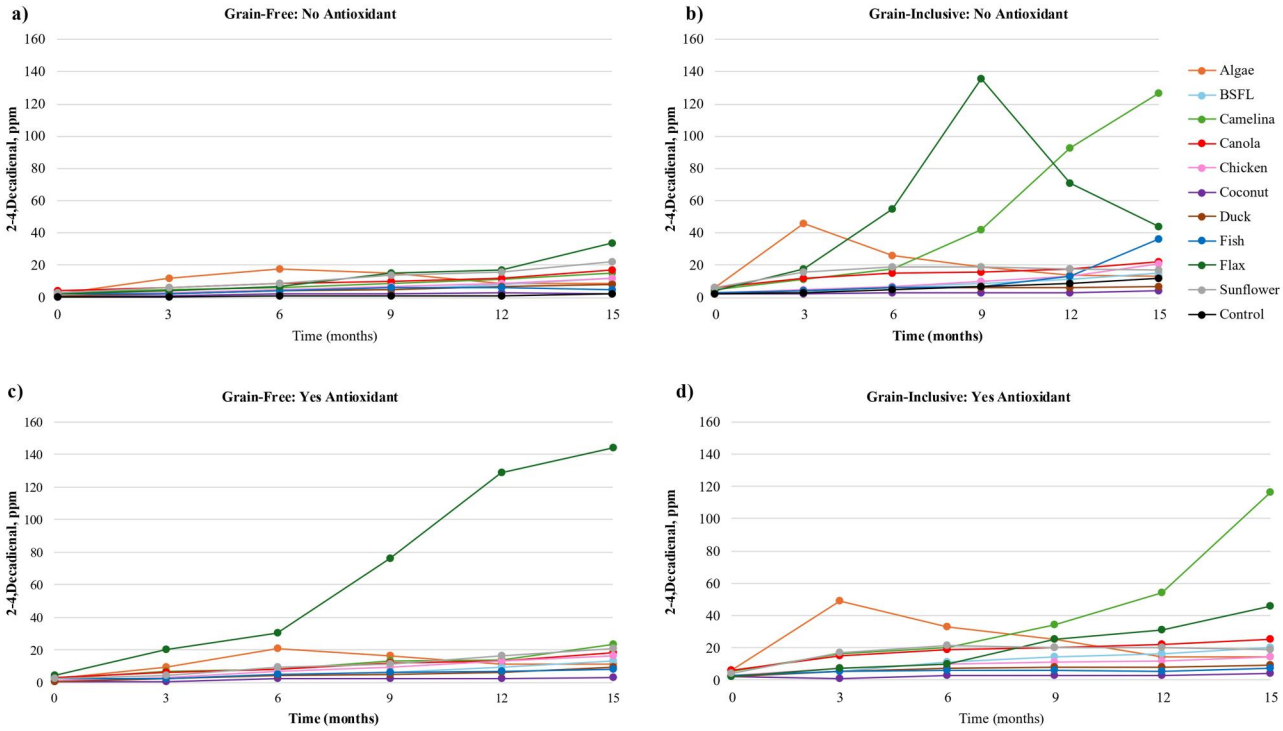


Figure 3 2,4-Decadienal concentrations (ppm) of extruded dog kibble coated with different lipid sources during 15 mo of ambient storage (22°C, 50% RH). Panels represent: (a) grain-free kibble without antioxidant inclusion, (b) grain-inclusive kibble without antioxidant inclusion, (c) grain-free kibble with antioxidant inclusion, and (d) grain-inclusive kibble with antioxidant inclusion.

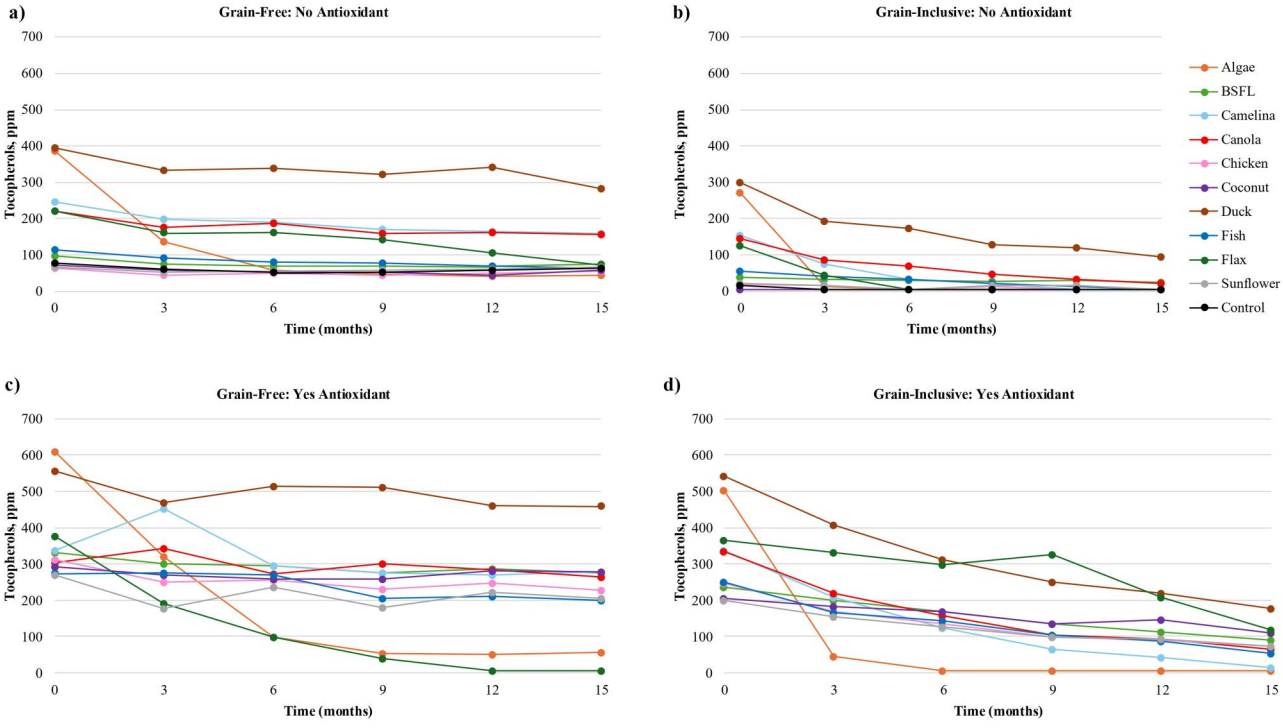


Figure 4 Tocopherol concentrations (ppm) of extruded dog kibble coated with different lipid sources during 15 mo of ambient storage (22°C, 50% RH). Panels represent: (a) grain-free kibble without antioxidant inclusion, (b) grain-inclusive kibble without antioxidant inclusion, (c) grain-free kibble with antioxidant inclusion, and (d) grain-inclusive kibble with antioxidant inclusion.

differences were not detected in PV between the two kibble types at 12 or 15 mo. In addition, there was no oil × antioxidant inclusion interaction observed ($P_{Oil \times Antioxidant} = 0.994$).

Secondary oxidation products (hexanal and decadienal)

Concentrations of hexanal and decadienal independently increased with time during ambient storage (Hexanal: $P_{Time} = 0.022$; Decadienal: $P_{Time} < 0.0001$). Grain-inclusive kibble had greater mean concentrations of both secondary aldehydes than grain-free kibbles (Hexanal: $P_{Kibble} < 0.0001$; Decadienal: $P_{Kibble} = 0.007$). A time × kibble interaction was present for hexanal concentrations ($P_{Time \times Kibble} = 0.027$), where grain-inclusive kibbles had greater concentrations of hexanal at each time point, as compared to grain-free. In particular, differences between kibble types were greater later in storage (9–15 mo). However, this interaction was not present with decadienal ($P_{Time \times Kibble} = 0.325$). Coating status affected decadienal concentrations ($P_{Coated} = 0.019$), with uncoated controls having lower concentrations compared with coated treatments, regardless of time. Coating status did not affect hexanal concentrations ($P_{Coated} = 0.819$). In addition, there were no time × coating interaction observed for either hexanal ($P_{Time \times Coated} = 0.992$), or decadienal ($P_{Time \times Coated} = 0.300$).

Within coated treatments, mean hexanal and decadienal concentrations differed between oil types (Hexanal: $P_{Oil} = 0.010$; Decadienal: $P_{Oil} < 0.0001$). Mean hexanal concentrations of algae oil were greater than BSFL, coconut, chicken, duck, fish, and sunflower. In comparison, mean decadienal concentrations were greater in algae, camelina and flax as compared to BSFL, coconut, duck, and fish. In addition, fish, BSFL, canola, chicken, and sunflower were all greater than coconut. Oil × time interactions were observed for both hexanal and decadienal concentrations (Hexanal: $P_{Time \times Oil} < 0.0001$; Decadienal: $P_{Time \times Oil} < 0.0001$). At baseline, hexanal concentrations did not differ among oils, whereas decadienal concentrations were greater for canola than BSFL, duck, and coconut. By 3 mo of storage, algae-coated samples had greater concentrations of both hexanal and decadienal compared with fish and flax for hexanal and BSFL, chicken, duck, and fish for decadienal. Hexanal concentrations at 6 and 9 mo remained greatest in the algae-coated samples, exceeding all other oils evaluated. However, decadienal concentrations were greatest in the flax-coated samples, exceeding BSFL, chicken, duck, and fish. Later in storage (12 and 15 mo), differences among oils were more restricted for hexanal, where the only differences in concentration were between algae and coconut, but differences remained pronounced for decadienal. Flax- and camelina-coated samples exhibited the greatest decadienal concentrations at these later time points, exceeding BSFL, coconut, duck, fish, and algae. Across all storage times and for both secondary aldehydes, coconut oil consistently had the lowest concentrations of all oils investigated.

Within coated treatments, grain-inclusive kibble had greater mean hexanal and decadienal concentrations than grain-free kibble (Hexanal: $P_{Kibble} = 0.0001$; Decadienal: $P_{Kibble} < 0.0001$). Although concentrations increased for both kibble types over time, concentrations of both secondary markers remained greater in the grain-inclusive kibble at 0–9 mo. There were no effects of antioxidant, time × antioxidant interaction and oil x antioxidant interaction on either hexanal or decadienal concentrations within coated treatments ($P > 0.10$).

Antioxidant (tocopherol) depletion

Within coated treatments, tocopherol concentrations declined over time under ambient storage conditions ($P_{Time} < 0.0001$). As expected, antioxidant inclusion affected tocopherol concentrations ($P_{Antioxidant} = 0.005$), with antioxidant-containing treatments maintaining greater tocopherol concentrations. However, there were no differences in tocopherol concentrations with antioxidant inclusion over time ($P_{Time \times Antioxidant} = 0.261$). Among oils, tocopherol concentrations decreased from baseline to endpoint in algae, camelina and flax oils ($P_{Time \times Oil} < 0.0001$), with no differences across time points in the other oil types analyzed. Mean tocopherol concentrations were lower in the grain-inclusive kibble as compared to the grain-free kibble ($P_{Kibble} < 0.0001$). Specifically, concentrations were lower at 3–15 mo in the grain-inclusive kibble as compared to grain-free kibble, although concentrations in both decreased over time ($P_{Time \times Kibble} < 0.0001$). There were no effects of time × antioxidant ($P_{Time \times Antioxidant} = 0.407$) and time × oil interactions ($P_{Time \times Oil} = 0.261$) on tocopherol concentrations during 15 mo ambient storage.

Oxidation rate (slope analysis)

All ambient samples ($n = 42$) had complete time series data at 0, 3, 6, 9, 12, and 15 months, allowing estimation of per-sample oxidation rates for each marker. Under ambient storage, the rate of PV increase did not differ between coated and uncoated samples ($P_{Coated} = 0.513$). However, within coated samples, oil type was associated with PV slope ($P_{Oil} = 0.0002$), with flax oil showing the greatest PV accumulation rate, followed by camelina oil. In comparison, algae and coconut had the lowest PV slopes of the oils assessed. There was a tendency for kibble type to affect PV slope, with the grain-free kibble tending toward greater rates of PV accumulation compared with grain-inclusive formulations ($P_{Kibble} = 0.083$). Antioxidant inclusion did not alter PV accumulation rates ($P_{Antioxidant} = 0.709$), and no oil × antioxidant interaction was detected ($P_{Oil \times Antioxidant} = 0.995$).

For secondary oxidation markers, rates of accumulation were not affected by coating status (Hexanal: $P_{Coated} = 0.887$; Decadienal, $P_{Coated} = 0.715$). Within coated treatments, oil type influenced the rate of decadienal accumulation ($P_{Oil} = 0.001$) and tended to influence the rate of hexanal accumulation ($P_{Oil} = 0.053$). Among oils, flax-, algae-, and fish-coated samples had the greatest rates of hexanal accumulation, whereas flax-, BSFL-, and camelina-coated samples had the greatest rates of decadienal accumulation. In contrast, coconut- and canola-coated samples had the lowest rates of accumulation for both secondary oxidation markers. Rates of hexanal accumulation under ambient storage were not affected by kibble type ($P_{Kibble} = 0.189$); however, grain-free kibble had a greater rate of decadienal accumulation compared with grain-inclusive kibble ($P_{Kibble} = 0.015$). Antioxidant inclusion and oil × antioxidant interaction were not associated with slopes of either hexanal or decadienal accumulation ($P > 0.10$).

Ambient depletion rates of tocopherols differed by oil type ($P_{Oil} = 0.007$) and kibble ($P_{Kibble} = 0.004$), but not by antioxidant inclusion ($P_{Antioxidant} = 0.756$) or oil × antioxidant interaction ($P_{Oil \times Antioxidant} = 0.994$). Tocopherol depletion was most rapid in

PUFA-rich oils, particularly flax and algae, whereas coconut and BSFL had the slowest depletion rates. The grain-inclusive diet had greater tocopherol loss over time compared with the grain-free diet. As expected, tocopherol slopes were negative across all treatments, reflecting progressive antioxidant depletion during storage.

Time to failure

Using the predefined failure criteria (PV $\geq$ 5 or hexanal + decadienal $\geq$ 25), 24 samples stored under ambient conditions reached time to failure by 15 mo. A summary of failure timing by treatment is provided in Table 2. Descriptively, the grain-inclusive algae-coated treatments were first to reach failure, which occurred at mo 0. The grain-free algae-coated and grain-inclusive PUFA-rich treatments (flax, camelina, sunflower, canola) had failures within 3–6 mo of ambient storage. In comparison, the coconut and duck treatments did not reach failure within the 15 mo storage period. The grain-inclusive diet was associated with shorter time to failure (P_{Kibble} = 0.001). In contrast, coating status, oil type, antioxidant inclusion and the oil × antioxidant interaction were not associated with time to failure ($P > 0.10$).

Double bond index

After accounting for kibble type and antioxidant inclusion, baseline DBI was positively associated with the hazard of oxidative failure ($P < 0.0001$). Each 0.1 unit increase in DBI was associated with a 30.5% greater hazard of reaching the predefined failure threshold (HR = 1.305; 95% CI: 1.157–1.471). There was no evidence of violation of the proportional hazards assumption for DBI, kibble type, or antioxidant inclusion ($P = 0.770$, 0.176, and 0.669, respectively).

Accelerated storage (3 mo)

Time to failure

Because samples were removed at failure, repeated-measures inference on oxidation markers over time was not performed for accelerated storage; time to failure was used as the primary endpoint. Under accelerated storage conditions, 22 samples reached the predefined failure criteria (PV $\geq$ 5 mEq/kg sample and/or hexanal + decadienal $\geq$ 25). A summary of failure timing by treatment is provided in Table 2. Descriptively, the grain-inclusive-algae and grain-free-algae treatments reached failure within 0 and 1 mo, respectively, of accelerated storage. Of the remaining samples that reached failure within the accelerated storage period, three were flax and four canola, with failures occurring between 1 and 3 mo. In contrast, the coconut and duck treatments did not reach the failure threshold within the 3 mo duration of accelerated storage. There was a tendency for grain-inclusive kibble to be associated with failure (P_{Kibble} = 0.065), but no effects of coating status, oil type, antioxidant inclusion, and oil × antioxidant interaction ($P > 0.10$).

Nutrient stability over ambient storage (15 mo)

Nutrient composition under ambient storage conditions is presented in Table 3.

Proximate analysis

Moisture content declined over the 15 mo storage period ($P_{Time} < 0.0001$). An effect of coating status was also observed for moisture (P_{Coated} = 0.022), with coated treatments having lower mean moisture compared with uncoated controls across time points. Between kibble types, the grain-inclusive kibble had a mean lower moisture content as compared to the grain-free (P_{Kibble} = 0.003). Specifically, at baseline, the moisture content of the grain-free kibble was greater than that of the grain-inclusive. However, over storage, moisture content decreased more for the grain-free kibble, resulting in no differences in moisture content between the two kibble types at 6 and 15 mo ($P_{TimexKibble} < 0.0001$). No time × coating status interaction was observed ($P_{Time \times Coated}$ = 0.249).

Protein concentration differed by coating status ($P_{Coated} < 0.0001$), with coated treatments having lower protein concentrations than uncoated controls. Protein was not affected by time (P_{Time} = 0.106), nor was a time × coating status interaction observed ($P_{Time \times Coated}$ = 0.924). The grain-free kibble had greater mean crude protein as compared to grain-inclusive ($P_{Kibble} < 0.0001$). Over storage, the grain-free kibble continued to have a greater protein concentration, however, crude protein concentration did not change within either kibble over the 15 mo storage period ($P_{Time \times Kibble}$ = 0.006).

As expected, fat concentration was greater in coated treatments ($P_{Coated} < 0.0001$) as compared to uncoated controls. In addition, the grain-inclusive kibble had a greater mean fat concentration as compared to grain-free kibble (P_{Kibble} = 0.0004). There were no effects of time (P_{Time} = 0.867, time × kibble interaction ($P_{TimexKibble}$ = 0.509) or time × coated interaction ($P_{TimexCoated}$ = 0.300) on fat content.

Similarly, ash concentration was affected by coating status ($P_{Coated} < 0.0001$), with coated treatments exhibiting lower ash concentrations compared with uncoated controls, but coating status did not affect ash over time ($P_{TimexCoated}$ = 0.415). Between kibble types, mean ash content was greater in the grain-free kibble ($P_{Kibble} < 0.0001$). Ash content did not change within either kibble over time, although the grain-free kibble consistently had greater ash content as compared to the grain-inclusive at each time point ($P_{TimexKibble}$ = 0.007).

Minerals

Kibble type influenced the concentration of all minerals evaluated ($P_{Kibble} < 0.0001$). Grain-inclusive diets contained greater concentrations of copper, magnesium, calcium, zinc, and phosphorus compared with grain-free diets, whereas grain-free diets contained greater concentrations of iron, potassium, sodium and manganese. There were no time × kibble interactions for any of the minerals analyzed ($P_{TimexKibble} > 0.10$).

Mineral concentrations of the finished coated and uncoated kibbles at 0, 6, and 15 months of storage are summarized in Table 4. Coating status affected copper, magnesium, calcium, phosphorus, and zinc concentrations ($P_{Coated} < 0.05$), with coated samples having lower concentrations than uncoated controls. A similar tendency was observed for iron and manganese (Iron: P_{Coated} = 0.071; Manganese: P_{Coated} = 0.071). In addition, time × coating interactions were detected for potassium ($P_{TimexCoated}$ = 0.009), magnesium ($P_{TimexCoated}$ = 0.011), calcium

Table 2 Time to failure under ambient (15 mo) and accelerated (3 mo) storage, defined as the time to reach a peroxide value (PV) of ≥ 5 mEq/kg sample or a sum of secondary aldehydes (hexanal + 2,4-decadienal) of ≥ 25 ppm, of grain-inclusive (grain) or grain-free extruded dog kibble coated in varying oils and fats at 10% or left uncoated (control), with or without the inclusion of an antioxidant (mixed tocopherols + rosemary extract) at 200 ppm/diet.

Oil/fat coating[a]	Kibble type	Antioxidant inclusion[b]	Time to failure (mo)[c]	
			Ambient[d]	Accelerated[e]
Algae	Grain	Yes	0	0
Algae	Grain	No	0	0
Algae	Grain-Free	Yes	3	1
Algae	Grain-Free	No	3	1
BSFL	Grain	Yes	9	N/A
BSFL	Grain	No	15	3
BSFL	Grain-Free	Yes	N/A	N/A
BSFL	Grain-Free	No	N/A	3
Camelina	Grain	Yes	6	1
Camelina	Grain	No	6	2
Camelina	Grain-Free	Yes	15	N/A
Camelina	Grain-Free	No	N/A	N/A
Canola	Grain	Yes	6	1
Canola	Grain	No	6	1
Canola	Grain-Free	Yes	15	3
Canola	Grain-Free	No	N/A	3
Chicken	Grain	Yes	15	N/A
Chicken	Grain	No	12	3
Chicken	Grain-Free	Yes	N/A	N/A
Chicken	Grain-Free	No	N/A	N/A
Coconut	Grain	Yes	N/A	N/A
Coconut	Grain	No	N/A	N/A
Coconut	Grain-Free	Yes	N/A	N/A
Coconut	Grain-Free	No	N/A	N/A
Control	Grain	No	9	3
Control	Grain-Free	No	N/A	N/A
Duck	Grain	Yes	N/A	3
Duck	Grain	No	N/A	N/A
Duck	Grain-Free	Yes	N/A	N/A
Duck	Grain-Free	No	N/A	N/A
Fish	Grain	Yes	N/A	N/A
Fish	Grain	No	12	2
Fish	Grain-Free	Yes	N/A	N/A
Fish	Grain-Free	No	N/A	N/A
Flax	Grain	Yes	9	3
Flax	Grain	No	6	1
Flax	Grain-Free	Yes	3	1
Flax	Grain-Free	No	15	N/A
Sunflower	Grain	Yes	3	1
Sunflower	Grain	No	6	1
Sunflower	Grain-Free	Yes	15	N/A
Sunflower	Grain-Free	No	15	N/A

[a]Oils and fats were applied as an external coating onto the kibble at 10%.
[b]NATUROX® R30 Liquid (mixed tocopherols + rosemary extract), Kemin Nutrisurance Inc, Des Moines, IA, USA; Yes: Antioxidant included within oil/fat coating at 200 ppm/diet; No: no antioxidant applied within coating.
[c]Time to failure defined as first analyzed time to reach (PV) of ≥ 5 mEq/kg sample or a sum of secondary aldehydes (hexanal + 2,4-decadienal) of) ≥ 25 ppm; N/A: treatment did not reach failure within the allotted storage period using pre-defined failure criteria.
[d]Ambient storage: Treatments stored in plastic sandwich bags for 15 mo at 22°C and 50% relative humidity.
[e]Accelerated storage: Treatments stored in plastic sandwich bags for 3 mo at 37°C and 40% relative humidity.

Table 3 Proximate, mineral and fatty acid composition of the uncoated (control) extruded dog kibble bases (grain-inclusive and grain-free) at baseline (0 months) after 15 months of ambient storage. Nutrients expressed on a DMB, with the exception of moisture.

Nutrient	Grain-inclusive		Grain-free	
	0 mo	15 mo	0 mo	15 mo
Proximates				
Moisture, %	6.50	6.02	9.28	5.52
Crude protein, %	29.68	29.63	34.46	33.99
Crude fat, %	9.89	11.54	8.43	9.08
Ash, %	6.48	7.15	8.12	8.03
Minerals				
Calcium, %	1.79	1.65	2.00	1.77
Phosphorus, %	1.16	1.08	1.24	1.11
Potassium, %	0.45	0.47	0.81	0.80
Sodium, %	0.17	0.17	0.26	0.25
Magnesium, %	0.13	0.13	0.13	0.12
Iron, mg/kg	82.57	76.00	87.55	83.31
Copper, mg/kg	17.68	17.39	17.36	16.44
Manganese, mg/kg	23.21	22.41	16.98	15.19
Zinc, mg/kg	296.16	293.90	275.19	258.00
Fatty Acids				
SFA, %				
C12:0	3.21×10^{-3}	1.06×10^{-3}	3.31×10^{-3}	1.06×10^{-3}
C14:0	0.057	0.065	0.055	0.060
C16:0	2.10	1.72	1.85	1.56
C18:0	0.56	0.71	0.58	0.64
MUFA, %				
C16:1	0.34	0.30	0.34	0.28
C18:1	3.80	2.58	3.60	2.34
C20:1	0.057	0.016	0.052	0.015
C22:1	ND	0.015	ND	ND
PUFA, %				
n-3				
C18:3 (α-Linolenic Acid)	0.15	0.15	0.22	0.14
C20:5 (Eicosapentaenoic Acid)	0.0032	ND	0.0022	ND
C22:6 (Docosahexaenoic Acid)	0.032	0.012	0.010	0.011
n-6				
18:2 cis (Linoleic Acid)	2.46	1.39	1.96	1.26
18:2 trans	0.016	1.43	0.014	1.30
C18:3γ	0.0096	0.21	0.0099	0.19
C20:2	0.012	0.0064	0.013	0.0058
C20:3E	0.0011	0.0023	0.0011	0.0021
C20:3H	0.013	0.015	0.015	0.013
C20:4 (Arachidonic Acid)	0.056	0.019	0.055	0.017

($P_{\text{TimexCoated}}$ = 0.007), phosphorus ($P_{\text{TimexCoated}}$ = 0.002), sodium ($P_{\text{TimexCoated}}$ = 0.002), and zinc ($P_{\text{TimexCoated}}$ = 0.005). Concentrations of these minerals were greatest at 6 mo for both coated and uncoated treatments, although the magnitude and significance of change differed between them. Declines in concentration from 6 to 15 mo were observed for magnesium, calcium, phosphorus, and zinc in both coating groups. However, calcium, phosphorus, and zinc also decreased from 0 to 15 mo in coated treatments, whereas the corresponding 0 to 15 mo differences were not detected in uncoated treatments. Potassium increased from 0 to 6 in coated treatments, while no Tukey-adjusted differences were detected in uncoated treatments. No Tukey-adjusted differences over time were detected for sodium.

Fatty acids

Fatty acid composition of the finished coated kibbles at 0 and 15 months of storage is summarized in Table 5. Across fatty acid classes (SFA, MUFA, n-3 PUFA, n-6 PUFA, and total PUFA) and individual fatty acids (LA, AA, ALA, EPA, and DHA), oil type was the primary determinant of fatty acid composition ($P_{\text{Oil}} < 0.0001$), reflecting inherent differences in the fatty acid profiles of the oils and fats used for kibble coating. As expected, poultry-,

Table 4 Mineral composition of coated and uncoated extruded kibble matrices at 0, 6, and 15 months of storage (DMB), averaged across kibble type (grain-inclusive and grain-free) and antioxidant inclusion (with or without).

Minerals	Coating Status	Uncoated			P_{Time}	P_{Coated}	$P_{TimexCoated}$
		0 mo	6 mo	15 mo			
Calcium, %	Uncoated	$1.89 \pm 0.054^{a,b}$	1.98 ± 0.054^{a}	1.71 ± 0.054^{b}	<0.0001	<0.0001	0.007
	Coated	1.67 ± 0.012^{a}	1.69 ± 0.012^{a}	1.61 ± 0.012^{b}			
Phosphorus, %	Uncoated	$1.20 \pm 0.027^{a,b}$	1.28 ± 0.027^{a}	1.09 ± 0.027^{b}	<0.0001	<0.0001	0.002
	Coated	1.07 ± 0.006^{b}	1.09 ± 0.006^{a}	1.03 ± 0.006^{c}			
Potassium, %	Uncoated	0.63 ± 0.054	0.68 ± 0.054	0.64 ± 0.054	0.0002	0.271	0.009
	Coated	0.58 ± 0.012^{b}	0.59 ± 0.012^{a}	$0.59 \pm 0.012^{a,b}$			
Sodium, %	Uncoated	0.21 ± 0.014	0.22 ± 0.014	0.21 ± 0.014	0.030	0.108	0.002
	Coated	0.19 ± 0.003	0.19 ± 0.003	0.19 ± 0.003			
Magnesium, %	Uncoated	$0.13 \pm 0.002^{a,b}$	0.13 ± 0.002^{a}	0.12 ± 0.002^{b}	<0.0001	<0.0001	0.011
	Coated	$0.12 \pm 0.0004^{a,b}$	0.12 ± 0.0004^{a}	0.12 ± 0.0004^{b}			
Iron, mg/kg	Uncoated	85.06 ± 4.90	86.19 ± 4.90	79.66 ± 4.90	0.510	0.071	0.638
	Coated	78.50 ± 1.10	77.39 ± 1.10	77.01 ± 1.10			
Copper, mg/kg	Uncoated	17.52 ± 0.62	19.36 ± 0.62	16.91 ± 0.62	<0.0001	0.001	0.447
	Coated	16.34 ± 0.14	17.42 ± 0.14	15.97 ± 0.14			
Manganese, mg/kg	Uncoated	20.10 ± 1.01	20.62 ± 1.01	18.80 ± 1.01	<0.0001	0.074	0.084
	Coated	18.20 ± 0.23	18.35 ± 0.23	17.52 ± 0.23			
Zinc, mg/kg	Uncoated	285.67 ± 6.97^{b}	306.82 ± 6.97^{a}	275.95 ± 6.97^{b}	<0.0001	0.0006	0.005
	Coated	268.13 ± 1.56^{b}	274.39 ± 1.56^{a}	259.60 ± 1.56^{c}			

Values expressed as LSM ± SEM. Across a row, different lower case letter superscripts (a, b, c) represent significant difference within uncoated/coated treatments between time, where a *P*-value of less than 0.05 is considered significant.

BSFL-, and coconut-coated treatments contributed greater proportions of SFA and MUFA, whereas plant- and marine-derived oils contributed greater proportions of PUFA.

Saturated fatty acid concentrations were affected by time × oil interaction ($P_{Time\ x\ Oil} < 0.0001$), where SFA concentrations decreased from 0 to 15 mo for BSFL and coconut treatments, whereas there were no changes in SFA concentrations over storage with the other oils and fats.

Kibble type did not have a main effect on SFA concentration ($P_{Kibble} = 0.433$); however, a time × kibble interaction was present ($P_{Time\ x\ Kibble} = 0.011$), where SFA concentrations decreased over 15 mo storage in the grain-inclusive kibble, but not in the grain-free kibble. Antioxidant inclusion did not affect SFA concentrations ($P_{Antioxidant} = 0.252$) and did not interact with time ($P_{Time\ x\ Antioxidant} = 0.274$) or oil type ($P_{Oil\ x\ Antioxidant} = 0.797$).

Monounsaturated fatty acid concentrations were influenced by storage time, where concentrations decreased from 0 to 15 mo ($P_{Time} < 0.0001$), but this decrease over time was not associated with oil type ($P_{Time\ x\ Oil} = 0.113$), kibble type ($P_{Time\ x\ Kibble} = 0.255$), or antioxidant inclusion ($P_{Time\ x\ Antioxidant} = 0.330$). Additionally, there were no individual effects of kibble ($P_{Kibble} = 0.957$), antioxidant inclusion ($P_{Antioxidant} = 0.209$), or the interaction of oil type × antioxidant inclusion ($P_{Oil\ x\ Antioxidant} = 0.337$).

Polyunsaturated fatty acids

A time × oil interaction was observed for total PUFA concentrations ($P_{Time\ x\ Oil} = 0.001$), however, there were no differences observed in PUFA trajectories across oils after a Tukey Kramer post hoc adjustment. However, there was a tendency for total PUFA concentrations to decrease across 15 mo storage, regardless of oil/fat coating type ($P_{Time} = 0.073$). Kibble type ($P_{Kibble} = 0.817$), antioxidant inclusion ($P_{Antioxidant} = 0.998$) and their interactions did not affect total PUFA concentrations ($P_{Time\ x\ Kibble} = 0.900$; $P_{Time\ x\ Antioxidant} = 0.996$; $P_{Oil\ x\ Antioxidant} = 0.950$).

Total n-3 fatty acid concentrations were affected by time x oil interaction ($P_{Time\ x\ Oil} < 0.0001$). Oils enriched in n-3 fatty acids, including flax, camelina, fish, and algae oils, had the greatest declines over storage, whereas oils with lower baseline n-3 content had smaller or negligible changes. No differences in the magnitude of n-3 loss were observed between grain-inclusive and grain-free kibble ($P_{Time\ x\ Kibble} = 0.645$), or between treatments with and without antioxidants ($P_{Time\ x\ Antioxidant} = 0.872$). When assessed individually, ALA declined over storage ($P_{Time} < 0.0001$), regardless of kibble type and antioxidant inclusion ($P_{Time\ x\ Kibble} = 0.805$; $P_{Time\ x\ Antioxidant} = 0.895$). There was a tendency for a time × oil interaction ($P_{Time\ x\ Oil} = 0.069$), with oils rich in ALA (flax, camelina) having greater losses from 0 to 15 mo. There was a tendency for the grain-free kibble to have greater concentrations of ALA, as compared to the grain-inclusive ($P_{Kibble} = 0.058$). However, there were no effects of antioxidant or oil × antioxidant interaction ($P_{Antioxidant} = 0.906$; $P_{Oil\ x\ Antioxidant} = 0.919$). Storage time was associated with reductions in EPA and DHA concentrations ($P_{Time} < 0.0001$), with losses differing by oil type (EPA: $P_{Time\ x\ Oil} < 0.0001$; DHA: $P_{Time\ x\ Oil} = 0.006$). At baseline, algae- and fish-oil-coated treatments had similar EPA concentrations (0.63% and 0.65% DMB, respectively), whereas mean DHA concentration was approximately four-fold greater in algae—than fish oil coated treatments (1.64% vs. 0.39% DMB). Both of these treatments exhibited the greatest decline in concentrations over time, whereas oils containing minimal baseline EPA and DHA showed little change. Neither kibble type nor antioxidant inclusion influenced EPA or DHA concentrations, and no interactions with storage time were detected for these factors ($P > 0.05$).

Table 5 Fatty acid composition of extruded kibble matrices coated with different lipid sources at 0 and 15 mo of storage (% DMB), averaged across kibble type (grain-inclusive and grain-free) and antioxidant inclusion (with or without).

Fatty acid	Oil/fat coating	Time (mo)		P_{Time}	P_{Oil}	$P_{Time \times Oil}$
		0	15			
SFA, %	Algae	$4.10 \pm 0.28^{A,B,e}$	$3.95 \pm 0.27^{A,e}$	0.008	<0.0001	<0.0001
	BSFL	$7.42 \pm 0.50^{C,D,e}$	$4.71 \pm 0.32^{A,f}$			
	Camelina	$3.08 \pm 0.21^{A,e}$	$4.16 \pm 0.28^{A,e}$			
	Canola	$2.86 \pm 0.19^{A,e}$	$2.53 \pm 0.17^{B,e}$			
	Chicken	$4.99 \pm 0.34^{B,e}$	$4.33 \pm 0.29^{A,e}$			
	Coconut	$8.65 \pm 0.59^{D,e}$	$4.50 \pm 0.31^{A,f}$			
	Duck	$5.10 \pm 0.35^{B,C,e}$	$4.68 \pm 0.32^{A,e}$			
	Fish	$4.19 \pm 0.29^{A,B,e}$	$4.49 \pm 0.30^{A,e}$			
	Flax	$3.12 \pm 0.21^{A,e}$	$3.88 \pm 0.26^{A,e}$			
	Sunflower	$3.07 \pm 0.21^{A,e}$	$3.37 \pm 0.23^{A,B,e}$			
MUFA, %	Algae	3.50 ± 0.28	2.50 ± 0.20	<0.0001	<0.0001	0.113
	BSFL	4.75 ± 0.38	3.26 ± 0.26			
	Camelina	6.02 ± 0.48	4.63 ± 0.3			
	Canola	9.44 ± 0.75	4.73 ± 0.38			
	Chicken	7.30 ± 0.58	4.20 ± 0.33			
	Coconut	3.95 ± 0.31	2.80 ± 0.22			
	Duck	8.07 ± 0.64	5.23 ± 0.42			
	Fish	7.07 ± 0.56	3.57 ± 0.28			
	Flax	4.96 ± 0.39	3.07 ± 0.24			
	Sunflower	10.99 ± 0.87	5.35 ± 0.42			
PUFA, %	Algae	$4.62 \pm 0.94^{A,B}$	1.84 ± 0.38	0.073	0.001	0.037
	BSFL	$3.78 \pm 0.77^{A,B}$	4.44 ± 0.91			
	Camelina	$7.19 \pm 1.47^{A,B}$	5.22 ± 1.07			
	Canola	$4.41 \pm 0.90^{A,B}$	4.97 ± 1.02			
	Chicken	$3.92 \pm 0.80^{A,B}$	4.08 ± 0.83			
	Coconut	2.36 ± 0.48^{A}	2.09 ± 0.43			
	Duck	$3.21 \pm 0.66^{A,B}$	4.40 ± 0.90			
	Fish	$3.61 \pm 0.74^{A,B}$	1.85 ± 0.38			
	Flax	8.75 ± 1.79^{B}	4.80 ± 0.98			
	Sunflower	2.77 ± 0.57^{A}	3.01 ± 0.62			
n-3, %	Algae	$2.40 \pm 0.55^{A,B,e}$	$0.36 \pm 0.08^{A,B,f}$	<0.0001	<0.0001	0.0001
	BSFL	$0.28 \pm 0.06^{C,D,e}$	$0.18 \pm 0.04^{B,C,D,e}$			
	Camelina	$3.24 \pm 0.74^{A,e}$	$1.03 \pm 0.23^{A,f}$			
	Canola	$0.83 \pm 0.19^{B,C,e}$	$0.51 \pm 0.12^{A,D,e}$			
	Chicken	$0.25 \pm 0.06^{C,D,e}$	$0.14 \pm 0.03^{B,C,D,e}$			
	Coconut	$0.21 \pm 0.05^{D,e}$	$0.13 \pm 0.03^{B,C,e}$			
	Duck	$0.20 \pm 0.05^{D,e}$	$0.16 \pm 0.04^{B,C,D,e}$			
	Fish	$1.28 \pm 0.29^{A,B,e}$	$0.21 \pm 0.05^{B,C,D,f}$			
	Flax	$5.21 \pm 1.19^{A,e}$	$1.24 \pm 0.28^{A,f}$			
	Sunflower	$0.18 \pm 0.04^{D,e}$	$0.09 \pm 0.02^{C,e}$			
n-6, %	Algae	2.03 ± 0.40	1.38 ± 0.27	0.675	0.0008	0.712
	BSFL	3.50 ± 0.69	4.27 ± 0.84			
	Camelina	3.94 ± 0.77	4.19 ± 0.82			
	Canola	3.58 ± 0.70	4.46 ± 0.88			
	Chicken	3.67 ± 0.72	3.94 ± 0.77			
	Coconut	2.14 ± 0.42	1.95 ± 0.38			
	Duck	3.00 ± 0.59	4.24 ± 0.83			
	Fish	2.33 ± 0.46	1.63 ± 0.32			
	Flax	3.53 ± 0.69	3.50 ± 0.69			
	Sunflower	2.59 ± 0.51	2.92 ± 0.57			

(continued)

Table 5 (continued)

Fatty acid	Oil/fat coating	Time (mo)		P_{Time}	P_{Oil}	$P_{Time \times Oil}$
		0	15			
LA, %	Algae	1.79 ± 0.21	0.87 ± 0.10	<0.0001	<0.0001	0.285
	BSFL	3.38 ± 0.39	1.96 ± 0.23			
	Camelina	3.60 ± 0.41	1.51 ± 0.17			
	Canola	3.47 ± 0.40	1.83 ± 0.21			
	Chicken	3.49 ± 0.40	1.89 ± 0.22			
	Coconut	2.05 ± 0.24	1.17 ± 0.14			
	Duck	2.86 ± 0.33	2.02 ± 0.23			
	Fish	2.12 ± 0.24	1.05 ± 0.12			
	Flax	3.41 ± 0.39	1.17 ± 0.14			
	Sunflower	2.48 ± 0.29	1.40 ± 0.16			
ALA, %	Algae	0.13 ± 0.03	0.06 ± 0.01	<0.0001	<0.0001	0.069
	BSFL	0.26 ± 0.06	0.17 ± 0.04			
	Camelina	3.22 ± 0.72	1.02 ± 0.23			
	Canola	0.81 ± 0.18	0.50 ± 0.11			
	Chicken	0.22 ± 0.05	0.12 ± 0.03			
	Coconut	0.18 ± 0.04	0.12 ± 0.03			
	Duck	0.18 ± 0.04	0.14 ± 0.03			
	Fish	0.23 ± 0.05	0.10 ± 0.02			
	Flax	5.16 ± 1.15	0.95 ± 0.02			
	Sunflower	0.15 ± 0.03	0.07 ± 0.02			
AA, %	Algae	0.17 ± 0.03	0.05 ± 0.01	<0.0001	<0.0001	0.061
	BSFL	0.06 ± 0.01	0.02 ± 0.003			
	Camelina	0.05 ± 0.01	0.02 ± 0.003			
	Canola	0.05 ± 0.01	0.02 ± 0.003			
	Chicken	0.08 ± 0.01	0.04 ± 0.01			
	Coconut	0.05 ± 0.01	0.02 ± 0.003			
	Duck	0.07 ± 0.01	0.05 ± 0.01			
	Fish	0.09 ± 0.01	0.03 ± 0.004			
	Flax	0.05 ± 0.01	0.02 ± 0.003			
	Sunflower	0.05 ± 0.01	0.02 ± 0.003			
EPA, %	Algae	$0.63 \pm 0.17^{A,e}$	0.0031 ± 0.0010^{f}	<0.0001	<0.0001	<0.0001
	BSFL	0.0031 ± 0.0001^{B}	ND			
	Camelina	$0.0032 \pm 0.0009^{B,e}$	0.0020 ± 0.0007^{e}			
	Canola	$0.0026 \pm 0.0007^{B,e}$	0.0005 ± 0.0002^{e}			
	Chicken	0.0039 ± 0.0011^{B}	ND			
	Coconut	0.0039 ± 0.0011^{B}	ND			
	Duck	0.0024 ± 0.0007^{B}	ND			
	Fish	$0.65 \pm 0.18^{A,e}$	0.0051 ± 0.0030^{f}			
	Flax	$0.0008 \pm 0.0005^{B,e}$	0.0008 ± 0.0005^{e}			
	Sunflower	$0.0050 \pm 0.0014^{B,e}$	0.0050 ± 0.0001^{e}			
DHA, %	Algae	$1.64 \pm 0.71^{A,e}$	$0.30 \pm 0.13^{A,f}$	<0.0001	<0.0001	0.006
	BSFL	$0.015 \pm 0.006^{B,e}$	$0.0078 \pm 0.0034^{C,e}$			
	Camelina	$0.015 \pm 0.007^{B,e}$	$0.0044 \pm 0.0019^{C,f}$			
	Canola	$0.014 \pm 0.006^{B,e}$	$0.0104 \pm 0.0045^{B,e}$			
	Chicken	$0.017 \pm 0.007^{B,e}$	$0.01172 \pm 0.0051^{B,e}$			
	Coconut	$0.018 \pm 0.008^{B,e}$	$0.0078 \pm 0.0034^{C,e}$			
	Duck	$0.016 \pm 0.007^{B,e}$	$0.0192 \pm 0.0084^{B,e}$			
	Fish	$0.39 \pm 0.17^{A,e}$	$0.0910 \pm 0.0395^{A,B,f}$			
	Flax	$0.027 \pm 0.012^{B,e}$	$0.0167 \pm 0.0073^{B,e}$			
	Sunflower	$0.016 \pm 0.007^{B,e}$	$0.0095 \pm 0.0041^{B,e}$			

Values are expressed as back-transformed least square means (LSM) ± SEM. Statistical inference was conducted on log-transformed data. Down a column, different upper case letter superscripts (A–D), represent significant difference between oil/fat-coating within a time, where a *P*-value of less than 0.05 is considered significant; Across a row, different lower case letter superscripts (e, f), represent significant difference between treatments within an oil/fat-coating between time, where a *P*-value of less than 0.05 is considered significant; ND, non-detectable.

Total n-6 fatty acid concentrations were only affected by oil type (P_{Oil} = 0.0008), with camelina, canola and BSFL having the greatest total concentrations among oils and fats used. There were no effects of time (P_{Time} = 0.675), kibble (P_{Kibble} = 0.280), antioxidant ($P_{Antioxidant}$ = 0.950), or any two-way interactions ($P > 0.10$) on total n-6 concentrations. When assessed individually, concentrations of LA were affected by time ($P_{Time} < 0.0001$), as mean LA concentrations decreased during storage. However, there was no interaction of time with oil type ($P_{Time\ x\ Oil}$ = 0.285), kibble type ($P_{Time\ x\ Kibble}$ = 0.181), or antioxidant use ($P_{Time\ x\ Antioxidant}$ = 0.601). Similarly, there was no interaction of oil with antioxidant use ($P_{Oil\ x\ Antioxidant}$ = 0.789), or any individual effects of kibble type (P_{Kibble} = 0.196), or antioxidant inclusion ($P_{Antioxidant}$ = 0.631). Concentrations of AA similarly decreased during the 15 mo storage period ($P_{Time} < 0.0001$), and differed among oil/fat coatings ($P_{Oil} < 0.0001$). Specifically, AA concentrations were greater in algae-coated treatments than in BSFL-, camelina-, canola-, coconut-, fish-, flax-, and sunflower-coated treatments; duck-coated treatments had greater AA concentrations than camelina-, canola-, coconut-, and sunflower-coated treatments; and chicken-coated treatments had greater AA concentrations than sunflower-coated treatments. In addition, there was a tendency for a greater decline in AA in canola, algae and fish oils ($P_{Time \times Oil} = 0.061$), and a tendency for greater AA concentrations in the grain-free kibble, as compared to grain-inclusive (P_{Kibble} = 0.095). There were no effects of antioxidant inclusion ($P_{Antioxidant}$ = 0.990) or its interactions with time or oil ($P_{Time\ x\ Antioxidant}$ = 0.913; $P_{Oil\ x\ Antioxidant}$ = 0.798), and no effect of time x kibble interaction ($P_{Time\ x\ Kibble}$ = 0.720).

Discussion

To our knowledge, this is the first study to integrate oil source, kibble matrix, and antioxidant inclusion with multiple complementary endpoints, including primary and secondary oxidation markers, antioxidant depletion, fatty acid degradation, oxidation-rate estimates, and time to failure modeling. Lipid oxidation remains a central challenge in the formulation and storage of extruded pet foods, particularly as manufacturers increasingly incorporate oils rich in PUFA to support skin and coat health, mobility, and other functional outcomes (Roush et al. 2010; Vandeweerd et al. 2012; Corbee et al. 2013; Adler et al. 2018; Richards et al. 2023; Schäfer and Thom 2024; Kar et al. 2025). Collectively, the data presented herein provides a mechanistic and applied framework for interpreting oxidative stability across commercially relevant oil types, kibble formulations, and shelf life durations. These data further emphasize that careful consideration of the application of oils enriched in PUFA, especially EPA+DHA and AA, is warranted.

Across both ambient and accelerated storage conditions, oil type was the primary driver of oxidative behavior and time to failure, consistent with established relationships between fatty acid unsaturation and susceptibility to oxidation (Hołda and Głogowski 2016b; Kara 2021; Symoniuk et al. 2022; Larsen et al. 2025). Importantly, the additional analysis of baseline fatty acid composition demonstrated a strong association between degree of unsaturation and subsequent oxidative failure. After accounting for kibble type and antioxidant inclusion, each 0.1 unit increase in baseline DBI was associated with a 30.5% greater hazard of oxidative failure. This relationship supports degree of fatty acid unsaturation as an important contributor to the differences in oxidative stability observed among lipid sources. Oils enriched in long-chain n-3 PUFA, such as algae, flax, and camelina, had greater accumulation of primary (PV) and secondary (hexanal and 2,4-decadienal) oxidative products, steeper oxidation slopes, greater tocopherol depletion, and shorter times to failure compared with more saturated or moderately unsaturated fats, such as coconut oil and the poultry fats. These results align with the inherent chemical instability of highly unsaturated fatty acids, which contain multiple bis-allylic methylene groups that are particularly susceptible to hydrogen abstraction and radical propagation. As the number of double bonds increases, the activation energy for oxidation decreases, accelerating hydroperoxide formation and downstream fragmentation under even mild storage conditions (Tan et al. 2002; Parker et al. 2003; Martín-Polvillo et al. 2004).

Among the oils evaluated, algae oil exhibited the poorest oxidative stability, reaching predefined failure thresholds at or near the beginning of storage under both accelerated and ambient conditions. Notably, algae oil was not oxidized upon arrival to the manufacturing facility, yet oxidation markers increased within the short interval between coating application and subsequent analysis. This observation underscores an important practical point, in that oxidative deterioration can begin immediately after oil handling and surface application, even over short time frames. This emphasizes the need for increased control of oil exposure to oxygen, light, and heat during post-extrusion processing. This is particularly relevant for oils rich in n-3 fatty acids that are more prone to oxidation (Jung et al. 1989; Shahidi and Spurvey 1996; Aidos et al. 2002; Gharby et al. 2011; Pignitter et al. 2014). Although the algae oil was pretreated with tocopherols by the supplier, its elevated n-3 fatty acid (EPA and DHA) concentrations increased the susceptibility of the algae oil to oxidation beyond what the antioxidant could effectively mitigate under the present processing and storage conditions. Further research investigating an antioxidant better suited for n-3 fatty acid stabilization, increased dosage concentrations and/or the application of multiple synergistic antioxidants, for oils applied to extruded foods. Additionally, it is important to note that the application level of 10% in this article was done to equally compare across fat/oil treatments. Commercially, application of marine oils, with significant concentrations of EPA/DHA, would not typically exceed 4%–5%. Furthermore, internal inclusion of oils and fats into the kibble matrix was not investigated in this article and requires additional research to understand how it may impact the shelf life and oxidative stability of marine oils such as algae.

In contrast, the fish oil-coated treatments did not exhibit the same rate of oxidation as the algae-coated treatments. Only one fish-coated treatment reached failure during 15 mo ambient storage, while the remaining fish-coated treatments did not reach failure within this storage period. Despite containing similar EPA at baseline, differences in DHA concentrations at baseline between the algae- and fish-coated treatments may have contributed in part to this difference (Algae, 0 mo: EPA, 0.63 ± 0.17% DMB; DHA, 1.64 ± 0.71% DMB; Fish, 0 mo: EPA, 0.65 ± 0.18% DMB; DHA, 0.39 ± 0.17% DMB), as the fish oil treatments

contained a lesser concentration of DHA, as compared to the algae treatments. In addition, the fish oil treatments contained greater SFA than the algae oil and most plant oil treatments, which may have further contributed to improved stability by lowering the overall degree of unsaturation, consistent with the observed association between baseline DBI and oxidative failure. However, the difference in stability may have also been due to the upstream antioxidant pretreatment of the fish oil by the supplier before coating, which is common practice for commercial marine oils (Carvajal et al. 2014). Altogether, these results underscore that shelf life may be partially determined before a product enters storage, highlighting the importance of upstream oil sourcing, pretreatment, transport, and storage conditions before incorporation into finished diets. As such, all of these variables should be considered in the ingredient and product supply chain for commercial pet food production.

Duck and chicken fats had consistently high oxidative stability across both storage conditions, reinforcing their widespread use in the pet food industry as reliable lipid sources for extruded diets. Their stability reflects a fatty acid profile dominated by SFA and MUFA, thereby limiting susceptibility to radical initiation and propagation (Tan et al. 2002; Parker et al. 2003; Martín-Polvillo et al. 2004; Kara 2021). In this article, BSFL oil exhibited comparable behavior, with similarly low accumulation of oxidation products over time and limited failure during storage. The BSFL-coated treatments had SFA to a degree comparable to duck- and chicken-coated treatments, while also contributing LA. Although the fatty acid composition of BSFL oil is influenced by larval diet (Li et al. 2022), this is not unique to insect-derived fats, as the fatty acid profiles of poultry fats are likewise shaped by feed composition (Kanakri et al. 2018). Collectively, the oxidative stability of the BSFL treatments in this article suggests that BSFL oil performed similarly to conventional poultry fats under the conditions of this study, supporting its potential use in extruded pet food applications where extended shelf life is required. More broadly, these findings reinforce that lipid sources differ in oxidative susceptibility and may require different handling and stabilization strategies depending on their fatty acid composition.

Changes in fatty acid composition during ambient storage were consistent with the oxidation patterns observed in PV and aldehydes. Saturated fatty acids, which lack double bonds, remained comparatively stable over time, while PUFA declined across storage, with the greatest losses occurring in oils enriched in long-chain n-3 PUFA. These findings align with well-established oxidative hierarchies wherein susceptibility increases with the number of bis-allylic positions (Tan et al. 2002; Parker et al. 2003; Martín-Polvillo et al. 2004). In this article, decreases in EPA and DHA coincided with increases in secondary oxidation products, consistent with the conversion of hydroperoxides into aldehydes, ketones, and other reactive compounds (Chu et al. 2023). In the present study, mean concentrations of EPA and DHA, across all treatments, decreased 98.7% and 77.9%, respectively, during the 15 mo ambient storage period. This is consistent with previous work where EPA and DHA concentrations decreased by up to 33% in commercial extruded dog food after 12 mo of storage (Kara 2021), and complete loss of EPA and DHA in experimental extruded dog foods after 14 mo storage (Crosbie et al. 2024).

In the present study, evaluation of both PV and aldehydes clarified oxidation kinetics and emphasized the limitations of relying on PV alone as an indicator of oxidative progression. Peroxide value increased early in storage for PUFA-rich oils but became less discriminating later as hydroperoxides decomposed into secondary products. This pattern was most evident in algae-coated treatments, where PV declined after peaking while aldehydes remained elevated, consistent with progression from primary to secondary oxidation products (Liu et al. 2014). The investigation of secondary aldehydes as complementary markers to PV is well recognized, and their inclusion is particularly relevant to pet food as aldehydes are associated with sensory thresholds for rancidity that may affect palatability and consumer acceptance (Donfrancesco and Koppel 2017; Liu et al. 2014; Shields et al. 2025).

From a practical perspective, these findings have implications for strategies intended to deliver long-chain n-3 PUFA through dry extruded diets. Both EPA and DHA are frequently included for anti-inflammatory benefits (LeBlanc et al. 2008; Mehler et al. 2016; Carlisle et al. 2024); however, the rapid oxidation and deterioration observed herein reinforce the oxidative vulnerability of these fatty acids when applied externally to kibble under conditions intended to isolate and compare oil behavior. Importantly, this article used a high external application rate without an antioxidant included in the kibble matrix; therefore, the present results are not a direct representation of typical commercial formulations, but rather evidence that long-chain n-3 PUFA require careful stabilization when incorporated into extruded foods. Although clinical outcomes were not evaluated in the present study, losses of EPA and DHA during storage would reduce the amount of these fatty acids delivered to the animal at the time of feeding relative to their concentrations at manufacture. The biological relevance of this loss would depend on the initial dietary concentration, food intake, storage duration and conditions, and subsequent bioavailability. This is particularly relevant for diets formulated to provide EPA and DHA for specific functional purposes. Furthermore, preserving EPA and DHA during storage is only one consideration for nutrient delivery, as the delivery matrix (i.e., chew, oil or kibble) can also influence bioavailability and resulting plasma EPA and DHA concentrations in dogs (Goffin et al. 2017). Considerations to improve stability may include lower inclusion rates, optimization of fat application practices, incorporation of antioxidants within the broader formulation, use of a more targeted antioxidant system, protective packaging, and/or alternative dietary approaches for highly unsaturated oils. Plant-derived oils rich in ALA, such as flax and camelina, may offer an alternative n-3 strategy for dogs (Burron et al. 2024). In this article, flax-coated treatments still exhibited comparatively short times to failure, while camelina demonstrated intermediate stability. Future work should evaluate how oil selection, antioxidant systems, and delivery can be optimized to improve n-3 stability without compromising palatability, shelf life, or the health benefits of these n-3 PUFA for companion animals.

Antioxidant inclusion increased tocopherol concentrations at baseline; however, the antioxidant strategy applied here did not uniformly extend oxidative stability across all oil types. Historically, synthetic antioxidants such as ethoxyquin, butylated hydroxyanisole, and butylated hydroxytoluene have

provided robust oxidative protection in lipid-rich pet foods; however, regulatory changes and consumer-driven preferences have led to a broad industry shift toward natural antioxidant systems. While natural antioxidants are effective in many applications, they are generally less potent and may be depleted more rapidly under high oxidative pressure, particularly in PUFA-rich, surface-coated systems (Hilton 1989; Gross et al. 1994). In this article, tocopherol concentrations were progressively depleted during storage, with the greatest depletion in PUFA-rich systems (algae and flax), consistent with more rapid antioxidant consumption under higher oxidative pressure (Gross et al. 1994). Importantly, a single antioxidant type and inclusion level was intentionally applied across treatments to minimize formulation variability and isolate the effects of oil composition and kibble matrix. While the dose and type of antioxidant chosen are representative of common industry practice, this standardized approach limited the degree of protection achievable across oils with varying FA profiles. These findings support that antioxidant solutions for coated extruded diets are context-dependent and may require customization based on oil composition, coating load, and kibble matrix. Notably, the stability of the fish-oil treatments, despite low measured baseline tocopherols, suggests that upstream stabilization involved antioxidant components not captured by the present tocopherol assay. This may have included additional phenolic antioxidants, such as rosemary extract, and/or synergists such as ascorbyl palmitate (Schlieck et al. 2021; Jewell et al. 2024), reinforcing the relevance of supplier pretreatment practices to finished-product stability. Further research should optimize stability on an oil-by-oil basis with clear decision-making frameworks for food manufacturers.

Beyond oil composition, the kibble matrix itself also influenced oxidative behavior in the present study. Notably, the grain-inclusive kibble generally exhibited greater aldehyde concentrations, greater tocopherol depletion, and shorter time to failure under ambient storage than the grain-free formulation. However, the greater oxidative stability of the grain-free matrix in this article should not be interpreted as evidence that grain-free formulations are inherently more stable, as only one formulation of each matrix type was evaluated. The grain-inclusive diet contained greater concentrations of several minerals, including copper and zinc, while the grain-free diet contained greater iron. Transition metals, such as iron and copper, function as pro-oxidants and can catalyze lipid oxidation by accelerating hydroperoxide decomposition and promoting radical propagation (Mancuso et al. 1999). Differences in mineral availability, in addition to matrix-dependent properties such as moisture dynamics, water activity, porosity, and surface structure, may have contributed to the observed matrix effects by altering oxygen diffusion and lipid distribution within the coating layer (Pezzali and Aldrich 2019; Crosbie et al. 2024). Previous research reported that pulse-rich diets may require greater moisture input through steam during processing (Pezzali and Aldrich 2019), due to the hygroscopic nature of peas (Gujska et al. 1994), resulting in greater water activity and therefore lower shelf life (Crosbie et al. 2024). Although the grain-free diet did have a greater moisture content at time 0 as compared to the grain-inclusive (9.28% vs. 6.49%), that did not appear to impact the shelf life of this kibble when coated. However, a limitation of this article is that only moisture content was investigated over time and not water activity.

In addition, comparisons between ambient and accelerated storage revealed qualitatively consistent susceptibility hierarchies despite differences in temperature and sampling design. Algae- and flax-coated treatments consistently ranked among the least stable, whereas coconut- and animal fat-coated treatments generally exhibited greater stability and longer times to failure. Accelerated storage effectively identified high-risk oils and distinguished them from those that remained stable; however, quantitative shelf life values did not translate directly between conditions. This is consistent with the principle that elevated temperature changes oxidation kinetics and may not accurately reflect degradation behavior under normal storage environments, leading to differences in shelf life estimates between accelerated and ambient testing conditions (Velasco and Dobarganes 2002; Farhoosh 2007). Accordingly, accelerated testing may be most useful as a screening tool, whereas long-term ambient testing remains important for products labeled with a shelf life greater than 12 mo. In addition, these findings suggest that post-purchase storage conditions, including exposure to elevated temperatures in consumer environments, may further influence oxidative progression in PUFA-rich extruded diets and should be explored in subsequent design of appropriate storage tools and communication to the consumer to ensure animals receive the freshest food possible.

Several limitations warrant consideration for this article. First, only a subset of oxidative endpoints was assessed, and protein oxidation was not evaluated despite its relevance to product quality and potential interactions with lipid oxidation (Chanadang et al. 2016). Future research could consider including additional complementary chemical, physical, and sensory analyses to evaluate product and lipid quality more comprehensively. Such measures could include free fatty acids, p-anisidine value and thiobarbituric acid reactive substances (Liu et al. 2014). In addition, sensory analyses may be useful in understanding consumer acceptability through the detection of off-smells and color changes. Palatability studies could also be considered to capture differences in flavor and serve as a practical marker of oxidation progression (Shields et al. 2025). In addition, each treatment combination was represented by a limited number of experimental units per time point, reducing power to detect higher-order interactions among oil type, matrix, and antioxidant inclusion. Because nutrient analyses were conducted in singlet at each storage time point, the observed temporal changes in mineral concentrations, including increases at 6 mo in both coated and uncoated treatments and decreases from baseline to 15 mo for some minerals in coated treatments, may reflect sampling and/or analytical variability and should therefore be interpreted cautiously. Moreover, some oils were commercially pretreated with antioxidants before coating, which may have influenced their oxidative behavior. Although we aimed to source only oils and fats that were not pre-treated, this was not an option for many of the oils and fats within the present study. However, although this does represent a limitation for evenly assessing oxidative stability based on fatty acid profile, it is also reflective of the industry's commercial supply-chain realities and the oils and fats currently available to the pet food industry. Finally, we chose to store the samples in plastic

sandwich bags to minimize any effects of packaging on oxidative stability and shelf life, and to better isolate formulation effects. The results of this article may have been different if samples had been kept in nitrogen-flushed packaging systems as commonly used by the pet food industry, or other packaging specifically designed to protect the shelf life of pet food. Lastly, fatty acid profiles at baseline and endpoint were analyzed at different laboratories due to unforeseen analytical constraints, including equipment limitations at the time of baseline analysis. Although identical analytical methodologies were employed, minor variability related to instrumentation or personnel cannot be excluded.

In conclusion, of the factors assessed in this article, oil source had a major influence on oxidative stability in coated extruded pet foods, with the strong association between baseline DBI and time to oxidative failure supporting degree of fatty acid unsaturation as an important contributor to these oil-source differences. Kibble matrix, antioxidant strategy, and storage environment exerted additional meaningful effects. Integrating PV, aldehydes, tocopherol depletion, fatty acid losses, oxidation-rate estimates, and survival analysis provides a comprehensive interpretation of oxidation behavior and shelf life failure in externally coated extruded pet foods. Altogether, these findings underscore that PUFA-rich oils, especially those high in long-chain n-3 PUFA, are more susceptible to oxidation under the coating and storage conditions evaluated, highlighting the importance of oil-specific handling, coating, antioxidant and storage considerations. In contrast, more saturated or moderately unsaturated lipid sources were comparatively more stable under the conditions of this article.

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Author contributions

Alexandra Rankovic (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing—original draft), Anna K. Shoveller (Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing—review & editing), Jennifer Saunders-Blades (Conceptualization, Funding acquisition, Investigation, Methodology, Resources, Writing—review & editing), Sara Cutler (Conceptualization, Data curation, Investigation, Methodology, Resources, Writing—review & editing), Sarah MacDonald Murray (Investigation, Writing—review & editing), and Kathryn Lown (Investigation, Writing—review & editing)

Conflict of interest statement. A.R. and S.M.M. declare no conflicts of interest. A.K.S. is the Champion Petfoods Chair in Canine and Feline Nutrition, Physiology and Metabolism, consults for Champion Petfoods, was previously employed by P&G and Mars Pet Care, serves on the Scientific Advisory Board for Trouw Nutrition, and has received honoraria and research funding from various commodity groups, pet food manufacturers, and ingredient suppliers. At the time of submission, the following individuals are employed and affiliated with the following: K.L. and S.C. with Kemin Nutrisurance Inc, Des Moines, IA, USA; J.S-B. with Champion Petfoods Holdings, Edmonton, AB, Canada.

Data availability statement

The data that support the findings of the present research are available from the corresponding author upon reasonable request.

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