Effect of Nutritional Interventions on Longevity of Senior Cats

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ABSTRACT

The objective of this study was to evaluate whether antioxidants, alone or in combination with other nutritional supplements, increase health and longevity in a population of older cats. A group of 90 cats between the ages of 7 and 17 years was blocked into 3 groups by age, body condition score, and gender. Cats were assigned to 1 of 3 diets: control (basal diet of nutritionally complete cat food), basal diet with added antioxidants (vitamin E and β-carotene), and basal diet with added antioxidants, dried whole chicory root (source of prebiotic), and a blend of supplemental n-3 and n-6 fatty acids. The diets were fed exclusively for the remaining lifetime of each cat. Physical exams, body condition scores, complete blood count, serum chemistries, plasma fatty acids, serum antioxidant status, fecal microflora, urinalysis, and body composition by dual-energy x-ray absorptiometry were performed at study initiation and at periodic intervals thereafter. After 5 years, cats fed the diet with the antioxidants vitamin E and β-carotene, dried chicory root, and a blend of n-3 and n-6 fatty acids lived significantly longer than cats fed the control diet. Positive indicators of reduced disease incidence and improved intestinal health were also observed.

INTRODUCTION

In all mammals, aging is associated with changes in body condition, body composition, energy requirements, declining organ function and immune status, and other metabolic changes. Nutrition may play an important role in delaying such changes or preventing their progression.[1] In the older human population, a link between nutritional status, quality of life, and overall physical and cognitive health has been demonstrated.[2–5] Nutritional and healthy lifestyle modifications initiated in older human individuals were positively related to reduced mortality risk and delayed deterioration in health status, indicating that even health promotion at older ages can contribute to healthy aging.[6,7] In dogs, life-long maintenance of ideal body condition by calorie restriction delayed the onset of disease and extended median lifespan by 15% over full-fed littermates.[8]

Aging encompasses a complex set of processes that gradually leads to increased vulnerability and damage at the cellular and organ level, and eventual death of the organism. While multiple environmental and genetic factors may be involved, there is considerable evidence that oxidative stress plays a major pathophysiologic role in aging and may explain the pathogenesis of many age-related diseases. With increases in oxidative stress and reduced levels of circulating antioxidants, a potential link with

longevity would be expected. An increased requirement for dietary antioxidants in aging individuals is a logical conclusion.[9]

The influence of nutrition on the quality and length of life is of great interest to pet owners and veterinarians alike. The presumption that nutritional needs change with aging has been addressed through a number of approaches to the formulation of senior pet foods, but there are no published data to date showing that specific nutrient combinations, when given to healthy aging pets, increase longevity or delay the onset of disease.[10] There are no official guidelines for specific nutritional requirements in senior pets, nor is there consensus on when a pet becomes "senior." Nutritional therapy is widely practiced for aging pets with specific clinical problems such as renal disease,[11,12] cardiac disease,[13] diabetes,[14] and inflammatory bowel disease,[15] but in the clinically healthy aging pet, dietary modifications also may be useful to compensate for declining functional systems.

The primary objective of this study was to evaluate whether antioxidants, alone or in combination with other nutritional supplements, increase longevity in a population of older cats when compared with cats fed a nutritionally complete control diet. Two test diets were chosen, one with supplemental antioxidants vitamin E and β-carotene, and a second diet combining these same antioxidants with additional dietary modifications (a prebiotic and supplemental n-6 and n-3 fatty acids) that could have a positive impact on the health of cats. We hypothesized that combining several nutritional strategies could have synergistic or additive effects not evident in studies investigating only individual nutrient components, and that these potential nutrient interactions could measurably benefit the health and longevity of the aging cat. Initial results on longevity and several of the measured parameters are reported here.

MATERIALS AND METHODS

Animals and Housing

A multi-year feeding study was initiated in February 2000 with 90 healthy mixed-breed cats in a controlled research facility setting. Several age groups of senior cats were assigned to the trial in an attempt to positively influence the visible as well as physiological changes that occur during aging. The large age range provided an opportunity to study the effect of dietary manipulation initiated in mature as well as older adult animals.

Selected cats were at least 7 years of age, often considered to be the beginning of the senior life stage.[16–18] Cats were divided into 3 chronological age groups of 30 cats each: 7 to 9 years, 10 to 12 years, and 13 or more years. Initial ages ranged from 7 to 17 years. Each diet group had an equal distribution of the 3 age groups.

Prior to initiation of the study, cats were screened to eliminate those with pre-existing clinical disease. The screening consisted of complete physical exam by the colony veterinarian, complete blood count, serum chemistry, thyroid status, and urinalysis. Cats were also required to have normal fecal consistency (no loose stools) for inclusion in the study. A body condition score was assigned to each cat by the staff veterinarian using a numerical classification from extremely thin (1) to obese (5). Cats were distributed equally among dietary treatment groups on the basis of age, body condition score, and gender.

Cats were initially maintained based on the housing conditions to which they were most accustomed, either individually or in rooms of 25–27 cats. As group-housed cats became less active and unable to reach the elevated perches and feeding stations in therooms, they were moved to individualized housing to complete the study. Food was provided ad libitum with the exception of an overnight fast prior to anesthesia or blood collection. Water was available at all times. All housing was maintained at a room temperature of 18°C–24°C and 40%–50% relative humidity, with controlled lighting

to provide 12-hour light and dark periods. Cats were individually housed on days when feces were obtained for evaluation.

Diets

Cats were assigned to 1 of 3 diets: Diet 1 (control): nutritionally complete and balanced adult cat food; Diet 2: control formula + antioxidants (vitamin E as alpha tocopheryl acetate and β-carotene); and Diet 3: control formula + antioxidants + prebiotic (whole chicory root) + blend of oils (supplemental source of n-6 and n-3 fatty acids). Diets were produced every 6 to 8 weeks in the Nestlé Purina Product Technology Center (PTC, St. Joseph, Missouri, USA). Typical nutrient comparisons are shown in Table 1.

Animal Care and Health

Health monitoring and treatment of all animals were carried out according to established colony veterinary procedures throughout the trial. All cats were observed several times per day for general appearance and behavior by the veterinary and caretaker staff. Any cat exhibiting signs of distress or illness was thoroughly examined and a health report generated. Medical treatments were administered according to established colony veterinary procedures. Dietary treatments were not adjusted due to illness, and the dietary treatment group did not influence therapeutic regimens. All veterinary personnel were blinded to dietary treatment groups. Humane euthanasia was carried out according to established colony procedures only after all appropriate diagnostic procedures, therapeutic regimens, and multiple assessments failed to show a clinical response, and the staff veterinarian deemed the cat's prognosis poor.

Table 1. Nutrient Composition.

Nutrient	Diet 1 (Control)	Diet 2	Diet 3
ME, kcal/g DMB	4.8	4.8	4.8
Protein, % DMB	43.5	43.0	39.3
Fat, % DMB	35.6	35.9	36.9
Ash, % DMB	9.8	9.8	10.2
Calcium, g/1000 kcal	3.9	4.1	4.1
Phosphorus, g/1000 kcal	3.1	2.8	3.1
Vitamin E, IU/1000 kcal	69.9	140.7	149.5
β-Carotene, mg/1000 kcal	ND	5	5
Linoleic acid, % of dietary fat	10.5	10.5	21.3

ME = metabolizable energy;
DMB = dry matter basis;
ND = not detectable.

The test diets were fed as the exclusive source of nutrition for the remaining lifetime of each cat assigned to the trial. Fresh food was offered daily in stainless steel bowls. If a cat refused to eat the test diet and the lack of food consumption did not appear to be related to a medical condition, the cat was considered for removal from the study after all avenues to improve consumption were attempted.

Trial Protocol

The study protocol was reviewed and approved by the Nestlé Purina PTC Animal Care and Use Committee. Cats selected for the study were assigned to 3 groups of 30 cats per age level. Each group was divided equally between dietary treatments and started on trial over a 3-month period. If cats had to be removed from trial for low food consumption, replacement cats of comparable age, sex, and body condition were subsequently started on trial.

The following measurements were taken for all cats at study initiation: complete blood count, serum chemistries, plasmafatty acid profile (control and Diet 3 only), serum antioxidant status (superoxide dismutase, glutathione peroxidase, vitamin E, β-carotene), bone density and body composition by dual-energy x-ray absorptiometry (DEXA, GE Lunar Prodigy, GE Healthcare, Diegem, Belgium), fecal microflora (control and Diet 3 only), and urinalysis. Cats also were

given complete physical examinations and assigned a body condition score.

Food consumption was measured daily during the study, and body weights were assessed weekly. Clinical measurements taken at study initiation (time 0 [t0]) were repeated throughout the study duration, as shown in Table 2.

Routine veterinary dental prophylaxis was performed every 6 months, when cats were anesthetized for DEXA scanning. Anesthesia was performed according to established colony veterinary procedures, based on the health status of the cat. Urinalysis was performed as part of the routine health monitoring. Urine was evaluated using commercial reagent strips for chemical determinations, standard microscopic sediment analysis, Orion pH meter for urine pH (Model 620, Thermo Electron Corporation, Waltham, Massachusetts, USA) and Reichert Veterinary refractometer for urine specific gravity (Model 10436, Reichert Analytical Instruments, Depew, New York, USA).

Fresh fecal samples were collected and processed for microflora evaluation within 15 minutes of defecation. Samples (5 g) were mixed in a stomacher bag with 10% w/w glycerol, CO_2 flushed, and hot sealed. Samples were frozen at -80°C, and microflora analysis was performed in batches by phase within 3 months of collection. If a cat was placed on antibiotic therapy during the study, fecal collection was not performed

Table 2. Parameter Measurements and Frequency (in months).

Procedure	Cats	t0	3	6	9	12	15	18	21	24	27	30	33	39	42	45	48	51	54
Body condition score	All	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Veterinary physical exam	All	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Body composition (DEXA)	All *	X	X	X	X	X		X		X		X		X		X		X	
CBC/serum chemistry	All	X	X	X	X	X	X	X	X	X		X		X		X		X	
Plasma fatty acids	Diets 1 and 3	X	X	X	X									X					
Antioxidant enzymes SOD, GP	All	X	X	X	X	X		X		X		X		X		X		X	
Serum vitamin E	All	X	X	X	X					X		X		X		X		X	
Serum β-carotene	All	X	X	X	X														
Fecal microflora	Diets 1 and 3	X	X																
Urinalysis	All	X	X	X	X	X		X		X		X		X		X		X	

DEXA = dual-energy x-ray absorptiometry; CBC - complete blood count; SOD = superoxide dismutase; GP = glutathione peroxidase.

*In some cases, anesthesia and DEXA were not performed if the cat was considered by the veterinary staff to be too frail to undergo the procedure.

until at least 14 days after completion of antibiotic therapy. Fecal microflora (Bifidobacteria, Lactobacillus, *Clostridium perfringens*) were quantified using real-time polymerase chain reaction with Roche LightCycler® System (Idaho Technology Inc., Salt Lake City, Utah, USA).[19,20]

Blood samples were obtained by jugular venipuncture. For complete blood count and antioxidant enzymes, whole blood samples were collected in EDTA tubes and evaluated immediately using the Baker 9110 hematology analyzer (pre-2002; Bio Chem Immunosystems, Inc., Allentown, Pennsylvania, USA) or Sysmex KX-21N hematology analyzer (Sysmex America Inc., Mundelein, Illinois, USA) and Randox ELISA kits (Ransel and Ransod, Randox Lab, Crumlin, United Kingdom). For serum chemistry and vitamin analysis, serum was obtained within 30 minutes by centrifugation, and frozen at -80°C for later batch analysis by test phase. Serum chemistries were quantified on a Ciba Corning Express Plus analyzer (Bayer Healthcare, Tarrytown, New York, USA) through mid-2001, and a Hitachi 912 analyzer (Boehringer Mannheim Corporation, Indianapolis, Indiana, USA) since mid-2001. Samples for antioxidant vitamin analysis were sent to the Michigan State Diagnostic Center for Population and Animal Health, Nutrition Department.

Statistical Analysis

Analysis of variance was used to compare initial parameters across groups to confirm that randomization was effective in producing balance at baseline (t0) in the 3 study groups.

Using the number of days a cat was on trial until death, a Cox proportional hazards model was used to compare the survival rates of the 3 diets.[21] Because there was a wide range of ages of the cats at trial initiation, the initial age of the cat was used as a covariate in the model. Separate adjusted survival curves were generated for the 3 age groups of cats. The model used an initial age of 8, 11, and 14 years, representing the average initial age of cats in each age group. Hazard ratios along with their 95% confidence intervals were estimated.

To compare the average age at death for the cats on the 3 diets, a censored regression analysis was used.[22] In the censored regression, the dependent variable was the age of death and the independent variables were initial age and diet.

Analysis of measured parameters, such as blood values, body condition score, body composition by DEXA, body weight, and food consumption, was performed by a longitudinal analysis.[23] Results of longitudinal analysis were expressed as average slopes or "predicted means" (LSMEANS from PROC MIXED in SAS/STAT® Software, SAS Institute, Cary, North Carolina, USA) for cats with an initial age of 12 years. The longitudinal model allowed for each animal's trend to be considered over time and an average trend or slope predicted for each group. For parameters that were only measured at a few time points, repeated measures analysis of variance was performed.

Fecal microflora data was analyzed using Kruskal-Wallis test.[24]

Chi-square contingency table analysis was used to evaluate the incidence of pathologies at necropsy. In this analysis, 2-way contingency tables were used to determine the differences between Diets 1 and 3. Because most cats died with multiple pathologies, a separate analysis was performed for each group of pathologies. Cats that had not died at the time of analysis were not accounted for, so the analysis is considered preliminary.

All statistical calculations were performed using SAS.[25]

RESULTS

Study Population

A total of 15 cats were removed from the study for refusal to eat, mostly within the first 6 weeks of the trial. Ten of these cats were replaced with other cats and started on trial at a later date than the original 3 groups. Five cats had to be removed later in the study and were not replaced; 3 of those

cats (1 from each dietary treatment group) were removed by the veterinarian more than 6 months after trial initiation. These 3 cats have been included in the statistical data set and are considered "censored" data, along with the remaining cats that are still living.

Baseline Data (t0)

At the time of diet assignment, there were no statistical differences in clinical measures (age, weight), body composition (body condition score, DEXA parameters), hematological, or serum biochemical values between the 3 groups (Table 3). The analysis showed no significant differences between groups in any of the baseline measures, indicating that randomization was effective in producing balance at baseline in the 3 study groups.

Longevity

For the survival analysis, there were 88 cats in the data set: 68 had died, 3 were removed early, and 17 had not yet died at the time of analysis. Table 4 gives the results of the Cox proportional hazard model using initial age as a covariate. Results for the hazard ratios show that Diet 3 was significantly different than Diet 1 ($P < 0.01$). In this model, the hazard ratio of Diet 1 vs. Diet 3 was 0.39, meaning that the hazard of dying for the cats on Diet 3 was only 39% of the hazard of dying for the cats on Diet 1. There was no significant difference between Diets 1 and 2 or between Diets 2 and 3. Figures 1, 2, and 3 show the adjusted survival curves from the proportional hazard model using initial ages of 8, 11, and 14 years, respectively, which represent the average initial age of cats in each age group. For all starting ages, the analyses demonstrated that the proportion of cats surviving was significantly higher on Diet 3 than Diet 1 ($P < 0.05$).

The results of censored regressions comparing the diets for age at death are shown in Table 5. These results show that the cats on Diet 3 lived significantly longer than the cats on Diets 1 and 2. After calculating the predicted age of death for different initial ages, it was observed that cats on Diet 3 lived about 1 year longer than cats in the other groups (Table 6).

Disease Incidence/Pathology

Histopathology results from deceased cats were recorded and tabulated for 6 general disease pathologies: renal, pancreas, cancer of any type, thyroid (hyperplasia or adenomas), gastrointestinal, and liver. While the analysis must be considered preliminary because not all cats have died, there was a statistically significant difference for thyroid hyperplasia/adenomas ($P < 0.05$), with Diet 3 cats having significantly fewer incidences of disease. Only 14% of cats on Diet 3 were affected compared with 43.5% of cats on Diet 1. There was also a nearly significant trend for gastrointestinal pathologies (*P*

Table 3. Baseline Data (t0).

Variable	Diet 1	Diet 2	Diet 3	*P*-Value
Initial age (years)	11.7 ± 2.5	12.3 ± 2.4	12.2 ± 2.6	0.589
Initial weight (g)	4529 ± 1411	4118 ± 1195	4032 ± 993	0.254
Body condition score	2.62 ± 1.0	2.42 ± 0.7	2.64 ± 0.6	0.475
Body fat (%)	17.5 ± 12.1	14.9 ± 9.5	12.9 ± 8.2	0.246
Body fat (g)	817 ± 792	591 ± 469	501 ± 421	0.117
Lean mass (%)	79.7 ± 11.5	82.2 ± 8.9	84.1 ± 7.6	0.227
Lean mass (g)	3164 ± 793	2933 ± 774	2969 ± 627	0.453
RBC ($\times 10^6$ U)	8.86 ± 1.6	8.28 ± 1.7	8.87 ± 1.4	0.254
Albumin (g/dL)	3.34 ± 0.4	3.22 ± 0.5	3.34 ± 0.3	0.433
SUN (mg/dL)	23.5 ± 8.9	27.9 ± 14.0	25.4 ± 9.3	0.320
Calcium (mg/dL)	9.13 ± 1.0	9.15 ± 1.0	9.25 ± 1.2	0.894
Creatinine (mg/dL)	1.42 ± 0.5	1.34 ± 0.4	1.47 ± 0.4	0.467
Potassium (mEq/L)	4.80 ± 0.7	4.85 ± 0.5	5.13 ± 0.9	0.201
SUN:Creatinine ratio	16.8 ± 4.0	19.9 ± 9.0	17.3 ± 4.9	0.135
Vitamin E (µg/mL)	13.3 ± 8.3	10.6 ± 5.9	12.9 ± 4.8	0.244

RBC = red blood cell; SUN = serum urea nitrogen.

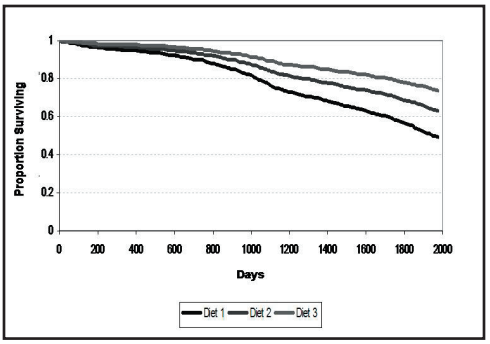

Figure 1. Adjusted survival curves starting at age 8 years.

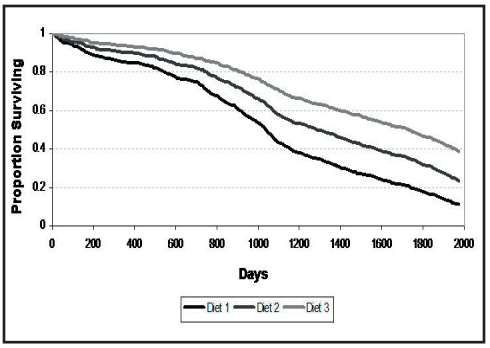

Figure 2. Adjusted survival curves starting at age 11 years.

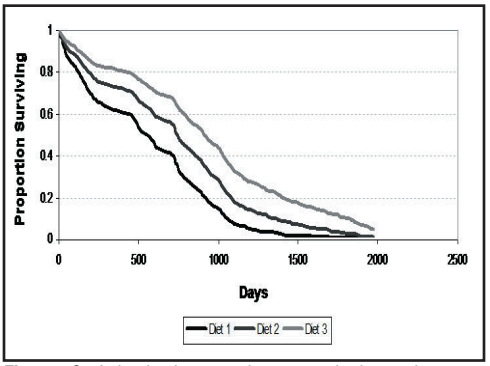

Figure 3. Adjusted survival curves starting at age 14 years.

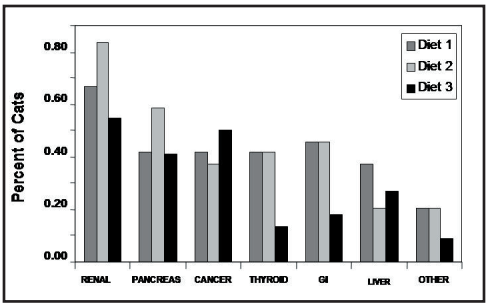

Figure 4. Incidence of pathologies in deceased cats.

= 0.08), with only 19% of cats on Diet 3 having some form of gastrointestinal disease compared with 43.5% of cats on Diet 1. For the other general pathologies, there were no statistically significant differences between diets (Figure 4).

Body Weight Maintenance

All 3 groups lost weight over time, on average, an expected result with aging cats. Dietary differences in the average trends (slopes) were statistically significant for body weight ($P < 0.05$), with cats fed Diet 3 showing less decrease in body weight over time than cats on Diets 1 and 2 (Figure 5). Average food consumption in calories per kilogram of body weight increased over time for all 3 groups. There was a statistically significant difference between the average trends (slopes) for food consumption ($P < 0.05$). Cats fed Diet 3 showed less of an increase in food consumption over time than cats fed Diets 1 and 2 (Figure 6).

Fecal Microflora

Initial and 3-month fecal microflora results are summarized in Table 7. There was a significant increase in Bifidobacteria ($P < 0.01$) and a significant decrease in *Clostridium perfringens* bacteria ($P < 0.01$) for the cats on Diet 3, while cats consuming Diet 1 had no significant changes. Cats on Diet 3 had an overall improved fecal flora profile than cats on Diet 1, as shown by the ratio of Bifidobacteria + Lactobacilli to *Clostridium perfringens*. From the Kruskal-Wallis test, the change from initial values was significantly different between the 2 diets for Bifidobacteria, *Clostridium perfringens*, and ratio of Bifidobacteria + Lactobacilli to *Clostridium perfringens* ($P < 0.01$). A total of 77% of cats on Diet 3 responded with either an increase in Bifidobacteria or Lactobacillus and/or a decrease in the number of *Clostridium perfringens* bacteria. Overall, the data showed that cats consuming Diet 3 harbored healthier gut microflora than cats fed the control (chicory-free) diet.

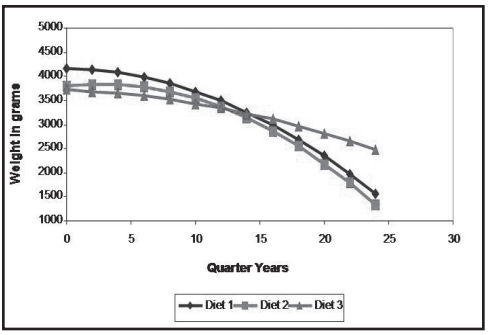

Figure 5. Body weights by diet (average trend lines or predicted means).

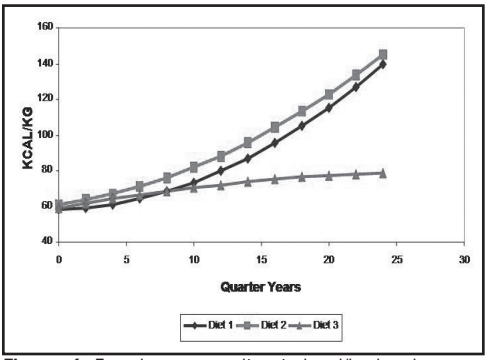

Figure 6. Food consumption in kcal/kg body weight (average trend lines or predicted means).

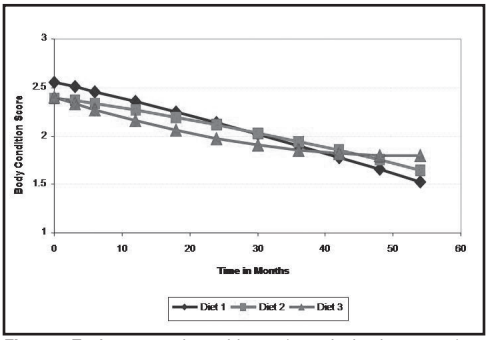

Figure 7. Average trend lines (predicted means) for body condition scores.

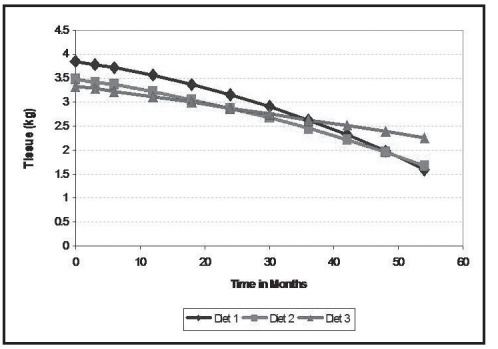

Figure 8. Average trend lines (predicted means) for total tissue (kg).

Body Composition and Body Condition Scores

For all parameters of body composition, including those measured by DEXA as well as body condition scores assigned by the examining veterinarian, longitudinal analysis showed significant decreases over time across all treatment groups ($P < 0.05$). Dietary differences in the average trends (slopes) were statistically significant ($P < 0.05$) for body condition score, lean body mass, bone density, bone mineral content, and total tissue (lean plus fat). Cats fed Diet 3 showed less decrease over time than both Diets 1 and 2 for body condition score (Figure 7), bone density, and bone mineral content, and less decrease over time than Diet 1 for lean body mass and total tissue (Figure 8). There were no significant differences between diets for fat (expressed as total fat, region fat, or tissue fat).

Blood

Cats fed Diet 3 had significantly higher levels of serum vitamin E, serum β-carotene, and plasma linoleic acid compared to cats fed the control diet. Tables 8 and 9 show the means and *P*-values from the ANOVA for β-carotene and linoleic acid. After 6 months on the study, cats on Diet 3 had significantly higher blood levels of β-carotene and linoleic acid than cats on Diet 1.

Vitamin E levels were evaluated at 0 (t0), 3, 6, 9, 24, 30, 36, 42, 48, and 54 months, and a longitudinal analysis of variance was performed relating vitamin E to time by diet. Figure 9 shows a plot of the predicted means for each diet for an initial

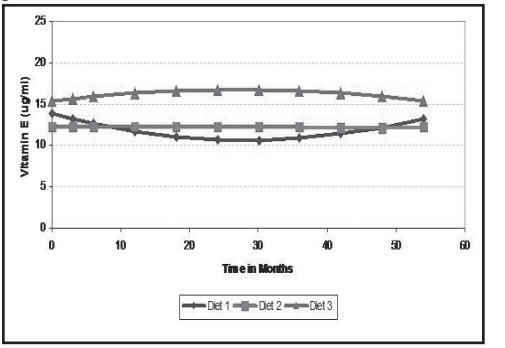

Figure 9. Average trend lines (predicted means) for serum vitamin E.

Table 4. Survival Analysis.

Variable	df	*P*-Value	Hazard Ratio	95% CI for Hazard Ratio
Diet 1 vs. Diet 2	1	0.2701	0.712	0.389–1.303
Age	1	<0.0001	1.464	1.253–1.711
Diet 1 vs. Diet 3	1	0.0042	0.386	0.201–0.741
Age	1	<0.0001	1.513	1.297–1.765
Diet 2 vs. Diet 3	1	0.2097	0.685	0.379–1.238
Age	1	<0.0001	1.382	1.197–1.595

df = degrees of freedom; CI = confidence interval.

age of 12 years. There was a significant diet-time interaction ($P < 0.05$), and Diet 3 cats had significantly higher serum levels of vitamin E than cats fed either Diets 1 or 2 ($P < 0.05$). The slopes of the linear and quadratic effects show that vitamin E levels did not change over time in cats fed Diet 2. For cats consuming Diet 1, vitamin E levels decreased with time, leveling off in later months. For those fed Diet 3, there was an increase in serum vitamin E with time but also a leveling off for the later months.

DISCUSSION

The hypothesis that a combination of nutritional interventions could have a positive effect on the health and longevity of senior cats was supported by the data reported here. Cats consuming a diet containing supplemental antioxidants, a source of prebiotics, and a blend of oils lived significantly longer than cats fed the control diet, when adjusted for their initial age. The hazard of dying for the cats on the supplemented diet was only 39% of the hazard of dying for the cats on the control diet. A key question is whether these results can be attributed to just one of the dietary manipulations or if they reflect an additive or synergistic effect of multiple nutritional interventions.

Caloric restriction has long been known as an intervention to increase longevity in a number of species.[8,26–29] While there are many theories, one mechanism involves a reduction in metabolic rate and reduced oxidative stress. Studies in both vertebrate and invertebrate species have shown that a variety of genetic and pharmacologic interventions can produce increases in longevity.[30] One study in mice showed that the life-extending effects of caloric restriction and the presence of a "longevity" gene that blocks the production or response to growth hormone were additive, suggesting that at least 2 mechanisms are involved.[31] Reducing total fat mass without caloric restriction also increased longevity in mice, suggesting that leanness rather than food restriction could be the key factor in extended longevity.[32]

While it is not known whether caloric restriction or the maintenance of a more lean body mass throughout life could extend lifespan in cats, there is evidence that extreme leanness in old cats may actually be detrimental. Emaciated cats had a significantly higher risk of death compared with cats in optimal body condition.[33] Perez-Camargo et al[34,35] demonstrated that body weight, lean body mass, and fat mass decline in cats over the age of 12 years, particularly in the last 1 to 2 years of life. Studies have also shown an increase in energy requirements despite progressive weight loss in

Table 5. Comparison of Diets for Age at Death.

Effect	df	Wald Chi Square	*P*-Value
All diets	2	6.7917	0.0335
Diet 1 vs. Diet 2	1	0.1222	0.7266
Diet 1 vs. Diet 3	1	6.8389	0.0089
Diet 2 vs. Diet 3	1	4.2744	0.0387
Initial age	1	35.9581	<0.0001

df = degrees of freedom

Table 6. Predicted Mean Age at Death at Different Initial Ages (Years).

Initial Age = 8		
Diet	**Predicted Mean**	**Standard Error**
1	12.9714	0.43624
2	13.1313	0.46382
3	14.0744	0.47951
Initial Age = 11		
Diet	**Predicted Mean**	**Standard Error**
1	14.3526	0.32643
2	14.5124	0.33221
3	15.4555	0.34803
Initial Age = 14		
Diet	**Predicted Mean**	**Standard Error**
1	15.7337	0.35903
2	15.8936	0.33421
3	16.8367	0.34411

aging cats.[36,37] Slowing down the inexorable body weight loss of aging could theoretically help extend the life expectancy of the elderly cat. In the current study, cats were fed ad libitum, and those on Diet 3 had better long-term maintenance of body weight, body condition, and body composition measures, such as lean body mass. They lived on average about a year longer than cats on the control diet.

A multi-factor nutritional approach to studying longevity is not uncommon. Recent population-based studies in humans have shown statistically significant increases in longevity for individuals eating a Mediterranean diet.[7,38] A combination of diet and healthy lifestyle factors showed a more than 50% reduction in mortality rate from all causes in elderly people who started the study between the ages of 70 and 90 years.[7] Thus, even in old age, it appears possible to positively affect longevity.

In this study, a group of mature and geriatric cats fed a diet consisting of supplemental antioxidants, a prebiotic, and a source of long-chain n-3 and n-6 polyunsaturated fatty acids lived significantly longer than cats on a control diet. Studies that have evaluated the influence of similar dietary modifications individually have shown various benefits.

Antioxidants

Antioxidants vitamin E and β-carotene were added to both test diets in this study since oxidative stress is thought to play a critical role in aging. Antioxidants are believed to exert a protective effect in cells by scavenging the toxic free radicals that cause oxidative stress and DNA damage.[9,29,39–42] Antioxidant status is reportedly reduced and may even be predictive of mortality in elderly humans.[39,40,43] However, high levels of circulating antioxidants have been seen in the "oldest old,"[40,44,45] providing a potential explanation for the extreme longevity of some individuals.

Vitamin E is a large component of plasma membranes and helps protect membrane phospholipids from peroxidation. Jewell et al[46] showed improved antioxidant status with

Table 7. Fecal Microflora (log cfu/g feces).*

	Diet 1 (Control)		**Diet 3**		***P*-Value, Change from Initial Diet 3 vs. Diet 1**
Bacteria	**Initial**	**3 Months**	**Initial**	**3 Months**	
Bifidobacteria (Bifido)	7.45	6.19	6.56[a]	8.23[b]	0.002
Clostridium perfringens	10.70	8.36	10.93[a]	7.70[b]	0.000
Lactobacilli (Lacto)	5.85	6.96	6.29	6.67	0.629
Bifido + Lacto:*Clostridium perfringens* ratio	1.24	1.57	1.17[a]	1.93[b]	0.007

*Means across rows with different letter superscripts are statistically different ($P < 0.05$).

high levels of dietary vitamin E (540 IU/kg) in dogs and cats. Vitamins E and C and β-carotene in combination reduced oxidative stress and DNA damage in lymphocytes in cats with renal insufficiency.[47] Vitamin E also has been reported to be efficient at preventing oxidation of low-density lipoproteins in humans.[48] At high doses (5 g/kg diet), aging male mice fed supplemental vitamin E showed a 40% increased median lifespan, a 17% increase in maximal lifespan, and up to 45% improved neurological performance over control mice.[49]

Epidemiological studies in humans have shown mixed results. A large 10-year study in women showed no significant effect of vitamin E on total mortality, but did show a significant reduction in cardiovascular mortality, particularly in women over the age of 65 years.[50] Other studies have demonstrated that a diet rich in antioxidants reduces the incidence of cancer and chronic disease, and the maintenance of a strong immune system may be a key factor.[9] In puppies, antioxidant supplementation improved immune response to vaccination,[51] and dietary lutein showed positive effects on the immune system in cats.[52] Antioxidant vitamins added to the diet also increased immunological responses of elderly people[53,54]; an inverse relationship between plasma vitamin E and infectious disease episodes was observed.[53] Similarly, supplementation with β-carotene reduced the number of infection days in an elderly human population.[55]

Assessment of antioxidant status in the living system is somewhat difficult, but a relatively simple indicator is the measurement of the specific antioxidants in the blood. Supplementation of vitamin E in a variety of diet types has been shown to increase serum vitamin E concentrations.[45,53,56,57] Chew et al[58] showed that cats are able to readily absorb β-carotene dosed orally in a water solution, with concentrations of plasma β-carotene increasing in a dose-dependent manner. Charlton et al[59] also showed significant increases in plasma β-carotene concentrations of cats after feeding a carotenoid-containing diet. Both moderate antioxidant supplementation and a diet high in carotenoids elevated serum carotenoids and antioxidant levels in an older human adult population.[60] The group eating more fruits and vegetables increased serum alpha tocopherol over time, and the authors speculated that perhaps concurrent supplementation with carotenoids may increase nutrient absorption or bioavailability of the tocopherols.

Both test diets in the current study were formulated with the same supplemental levels of vitamin E and β-carotene, and both groups of cats showed significant increases in serum β-carotene over control and baseline levels. However, only cats fed Diet 3 showed a significant increase in serum vitamin E over control and baseline values as well as a significant effect on longevity. Possible explanations could include higher levels of oxidative stress and antioxidant requirements in the Diet 2 cats, reduced absorption of vitamin E from the intestinal tract, or differences in the intestinal microenvironment, producing a vitamin E-sparing effect for Diet 3. The nature of the dietary lipids accompanying vitamin E and their intestinal lipolytic products may affect vitamin E absorption.[61] Alpha tocopherol absorption across enterocyte cell membranes is dependent on the incorporation and solubilization of the vitamin into mixed bile salt micelles.[62] Dietary long-chain polyunsaturated fatty acid levels have

Table 8. ANOVA for Plasma Linoleic Acid.

Parameter	Time	Diet 1	Diet 3	*P*-Values for Diet 1 vs. Diet 3
Plasma linoleic acid (g/100 g fat) ± SE	Initial	21.38 ± 0.5	20.04 ± 0.41	0.1738
	3 Months	19.89 ± 0.5	26.89 ± 0.41	<0.0001
	6 Months	19.17 ± 0.52	26.74 ± 0.43	<0.0001

SE = standard error.

Table 9. ANOVA for Serum β-Carotene.

Parameter	Time	Diet 1	Diet 2	Diet 3	*P*-Values		
					Diet 1 vs. Diet 2	Diet 1 vs. Diet 3	Diet 2 vs. Diet 3
Serum β-carotene (µg/mL) ± SE	Initial	0.004 ± 0.022	0.028 ± 0.021	0.018 ± 0.018	0.4312	0.6265	0.7214
	3 Months	0.067 ± 0.023	0.131 ± 0.021	0.146 ± 0.018	0.0413	0.0073	0.5799
	6 Months	0.036 ± 0.023	0.134 ± 0.021	0.171 ± 0.019	0.0021	<0.0001	0.1977

SE = standard error.

been shown to have an impact on vitamin E absorption that could be linked to a change in size and charge of the mixed micelles.[63,64] Wander et al[65] showed that a low n-6 to n-3 fatty acid ratio in the diet can reduce circulating vitamin E levels in dogs, presumably due to increases in lipid peroxidation caused by the higher levels of n-3 fatty acids. In pre-term human infants, however, formula supplemented with n-3 and n-6 polyunsaturated fatty acids resulted in higher plasma and cellular tocopherols than standard formulas.[66] The authors hypothesized that the fatty acids improve tocopherol solubility and stability in biological membranes.

Prebiotics

Diet 3 contained whole dried chicory root as a source of intestinal prebiotic. Prebiotic fibers are fermented in the colon and selectively stimulate the growth of Bifidobacteria and/or Lactobacilli, considered as "beneficial" bacteria of the large intestine. Inulin is an important source of fructo-oligosaccharide (FOS), and chicory root contains 50%–55% by weight inulin. Multiple studies have demonstrated the efficacy of chicory in modifying intestinal microflora in dogs and cats. Cats consuming food containing chicory had a significant increase in fecal Bifidobacteria and a significant decrease in fecal *Clostridium perfringens* compared with the same cats when fed control products without chicory.[67,68]

With advancing age, it is generally reported that Bifidobacteria are diminished in human feces, while *Clostridium perfringens* are increased.[69] Similar results have been observed in cats. Results from studies in young and old cats have shown a significant correlation between age and intestinal flora, with Bifidobacteria decreasing and *Clostridium perfringens* increasing with advancing age.[70] In older animals, positively modifying the intestinal ecosystem could have significant potential anti-inflammatory and immune benefits within the gut.[71,72] Chicory is also reported to have antioxidant properties, due to the presence of active compounds such as flavonoids and polyphenols that could contribute to improving health status of aging animals.[73]

In the current study, 77% of cats on Diet 3 responded with either an increase in Bifidobacteria or Lactobacillus and/or a decrease in *Clostridium perfringens*. These cats had an overall healthier intestinal microflora profile than those fed the control diet, as shown by the ratio of Bifidobacteria and Lactobacillus to *Clostridium perfringens*. In the long term, a reduction in inflammatory diseases of the gut could be expected. While preliminary, the analysis of the incidence of pathologies at necropsy in this study did show a trend for reduced numbers of cats on Diet 3 with histological evidence of gastrointestinal diseases.

In addition to positive effects within the gut, improving the balance of intestinal bacteria in aging cats could have systemic benefits. Reduction in toxic by-products of bacteria, such as *Clostridium perfringens*, could have far-reaching effects relating to

inflammation in a variety of body systems. More favorable bacterial fermentative end products could benefit renal health by reduced ammonia production and consequent reduced urea load on the kidneys.

Healthy cats without any signs of gastrointestinal tract dysfunction have been shown to have high numbers of bacteria, including *Clostridium perfringens*, in the proximal part of the small intestine.[74–76] This flora could be responsible for bacterial degradation of nutrients in the small intestine and for other side effects on digestion.[76] It has been demonstrated in broiler chickens that *Clostridium perfringens*, the primary small intestinal gram-positive bacteria in the chicken, have the ability to deconjugate bile salts by expressing high levels of bile salt hydrolase activity.[77] One consequence is an alteration of alpha tocopherol and lipid absorption, which can be corrected by antibiotic treatment.[78] When incorporated into the food, alpha tocopheryl acetate must be hydrolysed into alpha tocopherol prior to absorption. The main enzyme responsible is a pancreatic esterase that is dependent on bile salt in numerous species.[79–81] It could be hypothesized that the prebiotic source in Diet 3 decreased the population of *Clostridium perfringens* in the small intestine as well as in the large intestine, and therefore reduced the deconjugation of bile salts, their precipitation, and excretion into the feces. The better efficacy of pancreatic esterases by stronger bile salt activation could have led to increased vitamin E absorption with the prebiotic supplemented diet. Further studies would be needed to confirm this hypothesis.

Fatty Acids

An oil blend providing a source of polyunsaturated n-3 and n-6 fatty acids was included in Diet 3 for several reasons. The rate-limiting enzyme in fatty acid metabolism, delta-6-desaturase, reportedly declines in activity with age, while some pro-inflammatory metabolites of eicosanoids have been shown to increase with age,[82] suggesting that fatty acid requirements may be different in older pets. Additionally, polyunsaturated fatty acids have potential anti-inflammatory, skin and coat, and digestive health benefits.

The anti-inflammatory effects of n-3 fatty acids have been well studied.[83,84] The oxidation of fatty acids results in formation of eicosanoids, such as prostaglandins and leukotrienes, which have roles in the immune system, modulate inflammation, and maintain normal epidermal integrity. Reducing inflammation could be of significant benefit for numerous organ systems in the aging cat.

Older cats often exhibit changes in the appearance or consistency of the hair coat and skin that could be related to the lipid content of the diet. Essential fatty acid deficiency in cats results in numerous gross clinical, pathological, histological, and physiological changes. Clinical changes reported in cats fed essential fatty acid-deficient diets include a rough or greasy hair coat, dandruff or scaly skin, failure of wound healing, increased wax in ears, and increased susceptibility to infections.[85–89] MacDonald et al[90] noted a marked increase in water loss through the skin, alopecia, and focal exudative dermatitis in cats fed essential fatty acid-deficient diets. Adding essential fatty acids back into the diet reversed some of these negative effects.[83]

A number of digestive health benefits have been reported for the polyunsaturated fatty acids. Due to their anti-inflammatory and antibacterial properties, some polyunsaturated fatty acids have the ability to kill harmful bacteria that are likely to be present in the gastrointestinal tract.[91] Bomba et al[92] proposed that dietary fatty acids affect attachment sites of intestinal flora by modifying the fatty acid composition of the intestinal wall. Thus, dietary lipids may influence gastrointestinal microflora populations.

Nutrient digestibility, particularly fat digestibility, is reduced in old cats.[1,93,94] Recent studies evaluating cats in several research colonies fed a variety of dry and wet cat foods showed that the incidence of low fat digestibility increases with advancing age, affecting at least one third of geriatric cats

over 12 years of age.[34,95] There are likely multiple mechanisms involved, including subclinical diseases of the pancreas, liver, or intestinal mucosa, but defects in the absorptive capacity of the aging gut may also be associated.

Linoleic acid is an essential n-6 fatty acid in the diet of cats. The National Research Council (1986) recommended that all feline diets contain a minimum of 1 g of linoleic acid per 1000-kcal diet.[96] The Association of American Feed Control Officials Nutrient Profile for Cats lists the dietary requirement for linoleic acid in growing and adult cats as 1.25 g/1000 kcal ME.[97] While most commercial pet foods contain well over the minimum requirement, aging cats that suffer from reduced fat absorption or digestibility could be at risk for essential fatty acid deficiency, making the supplementation of higher levels potentially beneficial. One study of human cystic fibrosis patients found that increasing dietary linoleic acid helped improve fat malabsorption.[98]

CONCLUSIONS

Results from this study suggest that a diet supplemented with a combination of antioxidants, prebiotic, and long-chain polyunsaturated fatty acids, at specifically formulated levels, can increase lifespan in senior cats. It is logical to hypothesize that multiple nutritional approaches may be necessary to measurably slow the inevitable physiological decline of aging. Because increased oxidative stress and consequent disturbances in energy metabolism are probably critical mechanisms, both dietary interventions included elevated levels of antioxidants. An increased incidence of inflammatory conditions, renal disease, cancer, and disorders of intestinal function in the aging cat, as well as observed physical changes, such as a deterioration in skin and coat condition, led to the inclusion of prebiotics and dietary n-3 and n-6 fatty acids in the second test diet.

While these dietary interventions have shown various health benefits by themselves in other studies, until now there have been no reports indicating a measurable effect on lifespan in healthy old cats. Whether the results from this study reflect the combined benefits of the different dietary interventions or some synergistic effects between the nutrients is not known. Certainly there are some documented interactions between antioxidants such as vitamin E and polyunsaturated fatty acids that may have come into play in this study.[66,99–101] Additionally, the positive effects of chicory on gastrointestinal microflora and perhaps even improvements in vitamin E and fatty acid absorption may have played a role in this study.

In summary, senior cats fed a diet containing supplemental antioxidants vitamin E and β-carotene, dried chicory root, and a blend of n-3 and n-6 fatty acids lived significantly longer than cats fed a standard nutritionally complete feline diet. Positive trends for decreased incidence of thyroid and gastrointestinal pathologies suggest that the nutrient blend may provide some protection against certain disease states that may contribute to their increased longevity. Additional data collected over the next few years may help further elucidate the mechanisms for both the increased survival and improvement in health status of these aging cats.

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