Long-Term Feeding of DL-α Lipoic Acid to Dogs Is Safe

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ABSTRACT

Alpha-lipoic acid is well known as a covalently bound enzyme cofactor. In addition, lipoic acid has other cellular and molecular functions that have been explored in recent years, including its role as a powerful antioxidant. This study was designed to evaluate the safety of long-term oral exposure to DL-α-lipoic acid in dogs. Thirty healthy dogs (≥ 1 year; 15 male, 15 female) were enrolled in this randomized, placebo-controlled study for 12 months. The dogs were randomly assigned to one of five treatment foods with varying target inclusion levels of lipoic acid (0, 150, 1500, 3000, and 4500 ppm). Daily food intake, weekly body weights, monthly physical examinations, and monthly hematology and serum biochemistry were monitored to ensure the safety of the treatments.

Twenty-eight dogs completed the study. Food intake was highest for the food with the highest lipoic acid concentration. The average lipoic acid exposure for the different treatments groups were 0.31, 2.53, 26.3, 52.9, and 87.7 mg/kg body weight/day. Body weights did not differ between the treatment groups over time. There were statistically significant changes in certain blood parameters over time, but none of the trends were biologically significant, and the values stayed within or very close to the normal reference range. This study shows that long-term lipoic acid intake of up to 52.9 mg/kg body/day (3000 ppm diet) does not have any negative effects on the health of adult dogs.

INTRODUCTION

Alpha-lipoic acid is naturally found in mitochondria, where it plays a pivotal role in energy metabolism as a cofactor for α-ketoacid dehydrogenases (Reed 1974). The endogenous synthesis is assumed to provide sufficient lipoic acid for the intermediary metabolism. However, it has been suggested that it may be conditionally essential in aged animals (Ames 1998).

In addition lipoic acid can elicit other biological actions such as antioxidative, blood glucose regulation, and antiinflammatory. As powerful antioxidants, lipoic acid and its reduced form dihydrolipoic acid (DHLA) are capable of scavenging reactive oxygen species, regenerating vitamins C and E, and enhancing synthesis of endogenous antioxidants like glutathione, thus protecting cells from oxidative damage (Han et al. 1997; Packer et al. 1995). Both lipoic acid

and DHLA can chelate redox-active metals like Cu, Zn, Pb, and Fe (Ou et al. 1995; Suh et al. 2005).

In recent years, LA's role as direct acting antioxidants in vivo has been questioned because of its rapid clearance from blood following absorption and low accumulation in tissue. It has been hypothesized that lipoic acid acts through the modulation of signaling pathways and transcription to achieve the demonstrated in vivo antioxidant and antiinflammatory benefits (Petersen Shay et al. 2008). In Germany, lipoic acid has been approved as a drug to treat diabetic polyneuropathy and retinopathy for several decades. A number of studies have shown that treatment with lipoic acid results in significant improvement of neuropathic endpoints (Ametov et al. 2003; Ruhnau et al. 1999; Ziegler et al. 2006). In diabetic animals, lipoic acid has been shown to lower blood glucose through increased glucose uptake by skeletal muscles due to the enhanced translocation of the GLUT4 glucose transporter from its storage to the cell surface (Eason et al. 2002; Henriksen et al. 1997; Konrad et al. 2001). Lipoic acid helps regulate glucose metabolism in insulin resistant animals (Streeper et al. 1997). More recent work demonstrated lipoic acid's positive effects on vascular health and inflammation.

Through LA's stimulation of the PI3K/Akt signaling pathway and activation of endothelial nitric oxide synthase, lipoic acid elicits beneficial effects on vascular reactivity (Sola et al. 2005; Zhang et al. 2007). Bioavailability of lipoic acid in humans and dogs appears to be decreased if administered as a supplement concomitant with food or from a natural food source (Teichert et al. 1998; Zicker et al. 2010). Lipoic acid can be absorbed from the diet or supplements.

In humans about 20-40% of the administered dose is absorbed. Oral doses as high as 1,800 mg lipoic acid per day for six months administered to humans did not result in any serious adverse effects. An oral dose of 600 mg per day was found to have the best risk-to-benefit ratio (Ziegler et al. 2006; Ziegler et al. 1999).

Acute toxicity of lipoic acid varies among different animal species. Typical clinical signs of lipoic acid toxicity in dogs include vomiting, ataxia, tremors, seizures, hypersalivation, lethargy, and weakness (Loftin and Herold 2009). Cats are very sensitive to oral lipoic acid, with a reported maximum tolerated dose of 13 mg/kg body weight for a single oral dose (Hill et al. 2004). Packer, citing proprietary data, published the acute oral LD50 for dogs to be 400-500 mg/kg body weight (Packer et al. 1995). A recent case report of two dogs showed that clinical signs of acute toxicity and death can occur at estimated single oral doses of 190 mg/kg and 210 mg/kg body weight, respectively (Loftin and Herold 2009). This current study was designed to explore the safety of long-term, daily feeding of different dietary levels of lipoic acid to healthy adult dogs.

MATERIAL AND METHODS

The randomized, controlled study was approved by the Institutional Animal Care and Use Committee at Hill's Pet Nutrition, Inc. and conducted in accordance with all applicable Hill's animal welfare policies and procedures.

Animals and Food

Thirty healthy dogs (≥ 1 yr; 15 male, 15 female) of different breeds were selected to participate in this study (Table 1). Pregnant or lactating dogs were excluded from the study. The dogs were determined to be healthy by physical examination and by normal hematology and serum biochemical profiles.

The foods fed were formulated to meet or exceed nutrient recommendations for adult dogs in accordance with the Association of American Feed Control Officials (2000). The dietary treatments consisted of the control food with no added DL-α-lipoic acid (lipoic acid), and the control food plus the target inclusion levels for lipoic acid of 150 ppm, 1,500, ppm, 3,000 ppm, or 4,500 ppm on a dry matter base (DMB). The

Table 1. *Signalment of dogs*

ID Number	Sex	Breed	Pre-study Weight (Kg)	Age	Target levels of lipoic acid (DMB)
24637	Female	Beagle	12.4	2	Control - no lipoic acid added
25028	Female	Black & Tan Coonhound	15.6	1	
25898	Male	Mixed	16.9	3	
30329	Male	Beagle	18.0	1	
31927	Female	Mixed	15.4	2	
31977	Male	Beagle	11.4	3	
18661	Female	Mixed	15.8	2	150 ppm lipoic acid added
26051	Male	Beagle	13.8	4	
31258	Female	Treeing Walker Coonhound	13.8	1	
31662	Male	Basset Hound	19.9	1	
31921	Male	Mountain Cur	16.3	1	
32011	Female	Mountain Cur	12.7	3	
17087	Female	Beagle	15.9	1	1500 ppm lipoic acid added
17092	Female	Treeing Walker Coonhound	14.6	1	
17266	Male	Black & Tan Coonhound	16.8	1	
25321	Female	Mixed	11.6	1	
29674	Male	Catahoula Cur	18.9	2	
31720	Male	Basset Hound	13.9	2	
17422	Female	Shepherd	15.0	1	3000 ppm lipoic acid added
29680	Male	Labrador	16.3	1	
29687	Male	Mixed	17.9	2	
30899	Female	Treeing Walker Coonhound	11.7	1	
31976	Male	Beagle	11.7	2	
31997	Female	Mixed	13.5	1	
18563	Male	Treeing Walker Coonhound	16.3	3	4500 ppm lipoic acid added
18789	Female	Mixed	17.1	2	
29692	Male	Shepherd	16.1	1	
30901	Female	Treeing Walker Coonhound	13.4	1	
31153	Female	Mixed	10.1	1	
31669	Male	Blue Heeler	12.6	2	

foods had a metabolizable energy content of 3,535 kcal/kg.

Study Design

All dogs went through an acclimation period of 14 days during which their general health was assessed and baseline blood values established. During this period all of the dogs were fed the control food. Following the acclimation period, the dogs were blocked by gender and age and then randomly assigned to one of five dietary treatment groups. Fresh water was provided ad libitum. Dogs were fed enough food to

maintain their weight. For each individual dog, a maintenance energy requirement was calculated by multiplying the resting energy requirement (RER = 70 x body weight (kg) 0.75) by a factor of 1.6 to account for daily activity. To estimate the required daily food offering, the dog's requirement was divided by the metabolizable energy density of the food. Weekly body weights and daily food consumption were recorded.

Each week, food consumption and body weights were reviewed, and if necessary, the amount of food adjusted to maintain optimal body weight. Guidelines similar to AAFCO maintenance feeding trials were adopted to determine if animals should be excluded from the study. If weight loss exceeded 15% for any individual animal or 10% for any treatment group of animals, they would not be continued on study. Dogs were observed at least once daily by animal care technicians for any signs of illness or clinical signs of toxicity including decreased food consumption. Any suspected health problem was reported to the attending veterinarian and a physical examination was performed to assess the situation and institute treatment as needed. All observations were recorded.

During this 12-month study, the overall health of the dogs was evaluated by monthly physical examinations, hematology, and serum biochemistry profiles. Monthly values for hematology and serum biochemistry were compared to normal canine values to help determine overall health. Physical examinations evaluated the following systems: cardiovascular, neurological, muscular/skeletal, gastrointestinal, respiratory, hair/skin, behavior, general appearance, mucous membranes, eyes, ears, and teeth.

Food Analysis

The control and all test foods were analyzed for lipoic acid prior to start of the study to ensure that target supplementation levels were achieved. Lipoic acid was determined by a modified method of Witt et al (Witt and Rustow 1998; Zicker et al. 2002).

Blood Analysis

Fasting blood samples were obtained at baseline and then once a month for the duration of the study. Whole blood for serum biochemistry analysis was collected in a serum separator tube (with activator). The blood was allowed to clot and then centrifuged for 15 minutes at 1,200-1,500 rpm. The serum was removed using a disposable transfer pipette and aliquoted into two 2-mL polypropylene vials. One of the samples was frozen as a backup sample. The other was at kept in the refrigerator (40-60ºF) until analyzed using a Hitachi 912 analyzer (Roche Diagnostics, Florham Park, NJ) . Whole blood for complete blood count (CBC) analysis was collected into a evacuated blood collection tube containing potassium-EDTA. The whole blood samples were kept refrigerated (40-60ºF) following the collection until analyzed using a Serono-Baker System 9000 automated cell counter (Serono-Baker Diagnostics, Winchester, VA).

Statistical Analysis and Calculations

All analyses were performed using SAS Stastical Software version 9.2 (SAS Institute, Cary, NC). Because this was a safety study, data from all dogs were used in the statistical analysis, including the data from dogs that were dismissed during the course of the study. Significance was set at $p < 0.05$. Age and baseline weight data were analyzed using a one-way analysis of variance with dose as the only fixed effect.

Serum chemistry, CBC, and body weight data were analyzed by analysis of covariance with baseline (week 0) values. Age and body weight at the start of the study used as the covariates when they were statistically significant. Dose and time were fixed effects in the model. Because time is a continuous variable response, over time was analyzed using a linear random coefficients model. With the random coefficients model, fixed time and dose x time effects were included in the model. In addition, random subject and time x patient effects were included to allow the slopes and intercepts to vary randomly between subjects and account for random variation between the patients over time. Response over dose was analyzed

Table 2. *Demographics of study population.*

	Lipoic Acid Treatment Groups (PPM)					
Characteristics	**0**	**150**	**1500**	**3000**	**4500**	**p-value**
Age at start (years)	3.1 ± 1.43	2.8 ± 2.23	3.8 ± 1.33	2.33 ± 1.51	4.2 ± 2.64	0.74
Range	1 - 5	1 - 6	2 – 5	1 - 5	1 - 7	
Weight (kg)	14.9 ± 2.6	15.4 ± 2.6	15.3 ± 2.5	14.3 ± 2.5	14.2 ± 2.7	0.91
Range	11 - 18	13 - 20	12 - 19	12 – 16	10 - 17	
Sex						1.00
Male	3	3	3	3	3	
Female	3	3	3	3	3	

for linear, quadratic and cubic trends using orthogonal polynomials.

Because the levels of dose are unequally spaced coefficients for the orthogonal polynomials were calculated using the ORPOL function in PROC IML in SAS. Data for alkaline phosphatase , alanine aminotransferase, cholesterol, triglycerides, and total protein were found to be non-normally distributed, and a log transformation was performed prior to analysis.

Adverse events were categorized and analyzed by severity, food relationship, and body system affected. Contingency tables were created for each of the three categorical variables. Since severity and food relationship are ordinal variables, a Kruskal-Wallis test was used for analysis. Both variables were tested against dose levels. The null hypothesis being tested for the Kruskal-Wallis test was that all dose levels have the same distribution function with respect to median. The alternative hypothesis was that at least two of the dose levels have different distribution functions with respect to median. Body system affected was analyzed using a Fisher Exact test. The null hypothesis being tested for the Fisher Exact test was that there is no association between treatment and body system, and the alternative hypothesis was that there is an association between treatment and body system.

The lipoic acid content of the food on a DMB was calculated by adjusting the 'as is' analytical results by the moisture content as determined by the nutrient assays. The lipoic acid treatment group mean was calculated by dividing the sum of all assay results by the number of assays.

Intake of lipoic acid (mg/kg body weight) was calculated by using the mean food intake (g) times the lipoic acid content (ppm; as fed) divided by the animal's body weight (kg). Subsequently, a treatment group lipoic acid intake mean was also calculated.

Table 3. *Organ system affected by adverse events in the different treatment groups*

		System Affected				
Treatment	Count	Dermatological	Auditory	Gastrointestinal	Musculoskeletal	Oral
Control	4	1	2	0	1	0
150 ppm	2	0	0	2	0	0
1500 ppm	3	2	0	1	0	0
3000 ppm	2	1	0	0	0	1
4500 ppm	4	2	0	2	0	0

RESULTS

Dogs and Adverse Events

There were no statistically significant differences between treatment groups in age, body weight, and sex at baseline. (Table 2)

Nineteen adverse events were recorded during the study. Table 3 shows the organ systems that were affected by the adverse events. None of the adverse events were attributed to the food. Four of the nineteen events were due to parasites. There was no significant difference in severity of adverse events between treatment groups. The analysis of severity by dose level resulted in a p-value of 0.3824 indicating that all dose levels had the same distribution function. The analysis of adverse event relationship to food by dose level resulted in a p-value of 0.1014 indicating that there was no difference between the dose levels.

The analysis of body system by treatment resulted in a p-value of 0.0809, indicating that there was no association between treatment and the frequency of adverse events which occurred in body system. Two dogs (#31153 & #31997) were removed from the study for health reasons, one of them, #31997, died of heartworm infestation during the course of the study. The other, # 31153, was removed because of weight loss and leukocytosis.

Food Analysis

The composition of the five foods did not differ except for the concentration of lipoic acid. Table 4 shows the nutrient composition of the foods. Mean values for lipoic acid in the different treatment foods both on a DMB and as fed are shown in Table 5.

Food Intake

There was no significant dose main effect but a highly significant (p=0.0001) dose by month interaction. Plotting the monthly intake means for each dose indicates that the significant interaction resulted from the fact that the means vary from month-to-month. (Figure 1) However, there are two trends evident. In general, intake decreases over time, particularly at the lower lipoic acid doses. Secondly, intake is highest for the food with the highest lipoic acid concentration and decreases as the lipoic acid level decreases in the foods. The average lipoic acid exposure on a mg per kilogram body weight basis for each treatment group is shown in Table 5.

Body Weights

There were no statistically significant differences between treatment groups in body weight over time. The week by dose interaction had a p-value of 0.07. Table 6 shows the least square means (LSM) of the body weights at week 4, 13, 26, 39, and 52.

Hematology and Serum Biochemistry

Independent of the lipoic acid dose, statistically significant trends over time were observed for albumin, albumin:globulin ratio, serum creatinine, alkaline phosphatase, triglycerides, glucose, calcium, magnesium, potassium, chloride, phosphorous, bilirubin, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin volume, mean corpuscular volume, platelet count, red blood cells, and white blood cells. However, none of these trends were biologically relevant as the values stayed within or very close to the normal reference range (Table 7). Statistically significant interactions between the lipoic acid dose and time for albumin, albumin:globulin ratio, phosphorous, and hemoglobin were observed .

DISCUSSION

Daily consumption of a dry dog food containing up to 4,500 ppm lipoic acid for 12 months did not have any adverse effects on clinical appearance, food intake, body weight, or blood chemistry of healthy adult dogs. An interesting finding was that the food consumption per kg body weight was the highest in the group that was fed the food with the highest lipoic acid content (Figure 1). Despite the increased food consumption, the dogs did not gain any more weight than the dogs in the other treatment groups. This could potentially be explained by increased energy expenditure due to the high lipoic acid content in the food (Wang et

Table 4. *Nutrient composition of the study foods.*

Nutrient	Unit	Amount of Nutrient in the Control and Treatment Foods with different Levels of dl-alpha-Lipoic Acid (Mean ± SD)				
		Control (0 ppm)	150 ppm	1500 ppm	3000 ppm	4500 ppm
Moisture	%	7.5 ± 0.8	7.6 ± 0.8	7.9 ± 0.6	8.3 ± 0.5	8.2 ± 0.5
Protein	%	18.9 ± 0.3	18.2 ± 0.5	18.4 ± 0.4	18.6 ± 0.3	18.5 ± 0.2
Fat	%	15.3 ± 1.4	15.1 ± 0.2	15.5 ± 0.3	15.1 ± 0.4	15.0 ± 0.4
Crude Fiber	%	3.1 ± 0.1	3.1 ±0.2	3.4 ± 0.2	3.4 ± 0.2	3.2 ± 0.2
Ash	%	3.7 ± 0.3	3.6 ± 0.2	3.6 ± 0.1	3.7 ± 0.2	3.7 ± 0.2
Calcium	%	0.61 ± 0.07	0.60 ± 0.06	0.61 ± 0.06	0.62 ± 0.07	0.61 ± 0.08
Phosphorus	%	0.55 ± 0.05	0.52 ± 0.06	0.56 ± 0.06	0.55 ± 0.06	0.57 ± 0.05
Potassium	%	0.57 ± 0.02	0.54 ± 0.01	0.57 ± 0.02	0.55 ± 0.00	0.54 ± 0.01
Sodium	%	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.02	0.16 ± 0.01
Chloride	%	0.42 ± 0.01	0.31 ± 0.18	0.41 ± 0.02	0.43 ± 0.00	0.42 ± 0.00
Magnesium	%	0.12 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
Vitamin C	ppm	145 ± 19	135 ± 6	127 ± 15	122 ± 11	127 ± 8
Vitamin E	ppm	864 ± 245	1012 ± 150	933 ± 232	675 ± 305	728 ± 284

Table 5. *Lipoic acid concentration in the treatment foods and mean intake of lipoic acid*

Target (ppm; DMB)	Lipoic Acid concentration (PPM) analyzed by HPLC		Mean lipoic acid Intake (mg/kg body weight ± SE)
	Mean ± SD; DMB	Mean ± SD; as fed	
0	21 ± 20	19 ± 18	0.31 ± 0.01
150	157 ± 14	145 ± 13	2.53 ± 0.09
1500	1544 ± 112	1421 ± 107	26.3 ± 0.95
3000	3030 ± 214	2778 ± 194	52.9 ± 0.86
4500	4501 ± 556	4133 ± 512	87.7 ± 2.16

Table 6. *Body weight least square means (LSM) adjusted for baseline values.*

Dose (mg/kg)	Body Weights (kg; LSM ± SE)				
	Week 4	Week 13	Week 26	Week 39	Week 52
0	16.36 ± 0.25	16.33 ± 0.21	16.27 ± 0.21	16.22 ± 0.28	16.17 ± 0.38
2.5	16.30 ± 0.25	16.46 ± 0.21	16.70 ± 0.21	16.94 ± 0.28	17.17 ± 0.38
25	16.93 ± 0.25	16.84 ± 0.21	16.72 ± 0.21	16.59 ± 0.28	16.46 ± 0.38
50	14.96 ± 0.25	15.16 ± 0.21	15.45 ± 0.21	15.74 ± 0.28	16.04 ± 0.38
80	13.59 ± 0.25	13.81 ± 0.22	14.12 ± 0.23	14.44 ± 0.31	14.75 ± 0.42

Table 7. *Least square means of blood parameters at weeks 4 and 52.*

		Least Square Means (SE) of analytes in different treatment groups				
Blood analyte	**Wk**	**0**	**150**	**1500**	**3000**	**4500**
Albumin (g/dL)	4	3.41 (.07)	3.45 (.07)	3.43 (.07)	3.74 (.07)	3.60 (.07)
	52	3.05 (.08)	3.49 (.08)	3.38 (.08)	3.44 (.08)	3.49 (.09)
Albumin:Globulin Ratio	4	1.12 (.05)	1.07 (.05)	1.19 (.05)	1.22 (.05)	1.30 (.05)
	52	0.84 (.10)	1.18 (.10)	1.08 (.10)	0.92 (.10)	1.23 (.11)
Creatinine (mg/dL)	4	0.70 (.06)	0.92 (.06)	0.79 (.07)	0.68 (.07)	0.70 (.06)
	52	0.72 (.06)	1.02 (.06)	0.88 (.07)	0.76 (.07)	0.87 (.07)
Alkaline Phosphatase (IU/L)	4	64.5 (4.6)	62.3 (4.6)	45.1 (4.3)	53.6 (4.4)	50.4 (4.4)
	52	68.2 (4.8)	58.4 (4.6)	39.1 (4.2)	44.3 (4.3)	43.6 (4.3)
Triglycerides (mg/dL)	4	57.4 (4.4)	48.0 (4.2)	43.3 (4.1)	50.8 (4.3)	37.1 (3.9)
	52	66.8 (4.7)	56.0 (4.5)	60.3 (4.6)	58.3 (4.6)	38.5 (4.1)
Glucose (mg/dL)	4	79.7 (2.4)	82.8 (2.4)	91.2 (2.4)	78.1 (2.4)	91.0 (2.5)
	52	70.4 (2.9)	76.9 (2.9)	82.0 (2.9)	68.1(3.0)	78.7 (3.1)
Calcium (mg/dL)	4	11.3 (.10)	11.4 (.10)	11.2 (.10)	11.6 (.10)	11.3 (.11)
	52	10.4 (.11)	10.6 (.11)	10.4 (.11)	10.7 (.12)	10.6 (.12)
Magnesium (mmol/L)	4	1.61 (.03)	1.56 (.03)	1.52 (.03)	1.66 (.03)	1.70 (.03)
	52	1.86 (.04)	1.85 (.04)	1.81 (0.4)	1.88 (.04)	1.94 (.05)
Potassium (mmol/L)	4	4.71 (.06)	4.74 (.06)	4.47 (.06)	4.55 (.06)	4.44 (.06)
	52	4.93 (.07)	4.78 (.07)	4.73 (.07)	4.81 (.08)	4.50 (.08)
Chloride (mmol/L)	4	113.4 (.54)	111.5 (.58)	113.8 (.53)	112.7 (.56)	114.3 (.57)
	52	114.2 (.49)	114.0 (.53)	115.5 (.48)	113.7 (.55)	115.0 (.53)
Phosphorus (mg/dL)	4	3.98 (.22)	4.29 (.22)	3.84 (.22)	3.75 (.22)	4.18 (.22)
	52	4.07 (.16)	3.47 (.15)	3.38 (.16)	3.86 (15)	3.81 (.16)
Total Bilirubin (mg/dL)	4	0.14 (.01)	0.14 (.01)	0.13 (.01)	0.16 (.01)	0.16 (.01)
	52	0.23 (.02)	0.20 (.02)	0.21 (.02)	0.26 (.02)	0.24 (.02)
Hemoglobin (g/dL)	4	16.9 (.44)	16.7 (.44)	15.2 (.44)	17.1 (.44)	15.9 (.45)
	52	16.8 (.54)	17.7 (.55)	16.9 (.54)	17.2 (.56)	16.7 (.58)
MCH (pg)	4	23.6 (.20)	23.8 (.20)	23.9 (.20)	23.7 (.20)	23.6 (.21)
	52	24.9 (.34)	25.7 (.34)	25.4 (.34)	25.0 (.35)	25.9 (.37)
MCHC (g/dL)	4	33.1 (.23)	33.5 (.23)	33.3 (.23)	33.3 (.23)	33.3 (.24)
	52	34.2 (.30)	34.6 (.30)	34.3 (.30)	34.1 (.31)	34.7 (.32)
MCV (fl)	4	71.1 (.38)	70.9 (.37)	72.1 (.38)	71.2 (.38)	71.0 (.39)
	52	72.7 (.76)	74.3 (.76)	74.4 (.76)	73.2 (.77)	74.8 (.82)
Platelet Count (10^3/mm^3)	4	215 (20)	238 (19)	200 (20)	205 (19)	243 (20)
	52	215 (20)	206 (20)	182 (20)	179 (20)	168 (21)
Red Blood Cells (10^6/mm^3)	4	6.87 (.17)	7.01 (.16)	6.63 (.17)	7.26 (.16)	6.79 (.17)
	52	6.44 (.20)	6.88 (.19)	6.91 (.20)	7.00 (.20)	6.51 (.21)
White Blood Cells (10^3/mm^3)	4	13.6 (1.0)	10.9 (1.0)	11.2 (1.1)	12.7 (1.1)	13.9 (1.1)
	52	14.4 (.86)	10.4 (.85)	12.1 (.86)	14.1 (.87)	15.5 (.90)

al. 2010).

Typical clinical signs of lipoic acid toxicity in dogs include vomiting, ataxia, tremors, seizures, hypersalivation, lethargy, and weakness (Loftin and Herold 2009). Quick action following an accidental ingestion of a large dose of lipoic acid is paramount to avoid hepatic failure and acute renal failure. Of the 15 non-parasitic adverse events observed in this study, none of them was attributed to the food. Occasional vomiting occurred in five dogs; it was rated mild and was not associated with dose level. Daily observation of the dogs did not reveal any clinical signs of toxicity in any of the dogs during the course of the study.

Blood chemistry and hematology were monitored regularly throughout the study. Although statistically significant changes in certain blood parameters were observed, none of them were interpreted to be biologically significant. The values stayed within or very near the normal reference range of the laboratory. Since lipoic acid affects glucose metabolism and utilization in the body, hypoglycemia associated with other clinical signs could indicate toxicity following a large lipoic acid dose (Loftin and Herold 2009). We observed a statistically significant reduction in serum glucose concentrations during the year-long study in all treatment groups, including the Control group. All glucose values stayed within the normal reference range and furthermore, the control group showed the same trend which led to the conclusion that the apparent reduction in serum glucose concentrations was unrelated to lipoic acid dose present in the test groups. There was no time by dose interaction detected.

This study demonstrated that the inclusion of DL-α-lipoic acid of up to 3000 ppm into dog food does not present any health risk to dogs.

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