Nutrient intake and influence on markers of oxidative stress in zoo-managed male snow leopards (*Uncia uncia*)

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ABSTRACT: Oxidative stress (OS) results from the overproduction of reactive species. Nutrient intake can contribute positively or negatively to OS, and the lack of established nutrient requirements for most of the exotic species managed in zoos exacerbates the possibilities for nutrient imbalances that potentially could lead to reactive species production. The objective of this study was to evaluate the influence of nutrient intake and nutritional husbandry on markers of OS in male snow leopards (n = 14) maintained in U.S. facilities (n = 12). Diet samples and husbandry information were obtained and snow leopards were immobilized once for collection of blood. Samples were analyzed for chemical composition (diet and blood), antioxidant capacity (blood), and markers of OS (blood). Correlations between weekly nutrient intakes and markers of OS were analyzed by linear regression. Analyzed markers of OS included antioxidant enzymes (superoxide dismutase [SOD] and glutathione peroxidase [GPx]) and ferric reducing antioxidant potential that are protective against OS, and protein carbonyls, thiobarbituric acid reactive substances, and DNA/RNA damage that are indicative of oxidative damage. Weekly copper intake (10.1 to 80.2 mg) was negatively correlated with DNA/RNA damage (R^2 = 0.44; P = 0.01). Weekly sodium intake (4.4 to 12.7 g) was positively correlated with GPx activity (R^2 = 0.43; P = 0.04). More frequent feeding of whole prey (0.3 to 3 times/wk) was correlated with increased blood SOD activity (R^2 = 0.55; P < 0.01). In conclusion, greater dietary copper intake and more frequent feeding of whole prey may reduce OS in snow leopards. Dietary sodium intake and relationship with GPx activity should be further evaluated to determine benefit or detriment. No cause and effect can be inferred from our results, but our data suggest altering dietary form and nutrient concentrations may influence OS in snow leopards.

Key words: cat, diet, oxidative stress, snow leopard, whole prey

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INTRODUCTION

Snow leopards are listed as an endangered species by the International Union for Conservation of Nature, and zoo populations have been declining over the past 2 decades (Tetzloff et al., 2016). An improved understanding of nutrition in zoo-managed settings is vital to conserve an assurance population of the species (McCarthy et al., 2017). While the exact cause of the managed population decline is not known, dietary inadequacies could be a contributing factor to disease and abnormal

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physiologies, possibly through oxidative stress (OS). Oxidative stress is defined as a disturbance in the prooxidant–antioxidant balance, in favor of prooxidants or reactive oxygen species (ROS; Sies, 1985).

Animal diets contain many nutrients with both pro- and anti-oxidant properties. This includes some trace minerals and vitamins required for physiological function. Excesses of trace minerals may result in ROS production due to their redox potential (Tvrda et al., 2014). Antioxidants are employed to reduce and detoxify potentially damaging prooxidants and defend against oxidative damage. Antioxidants can be found in the body as enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which dismutate and neutralize ROS, or as nonenzymatic compounds in the diet, such as vitamins A and E, which donate electrons to neutralize ROS (Sies, 1997).

Effect of diet on OS has been evaluated in many of the domestic species, including poultry (Gao et al., 2010), swine (Lauridsen et al., 1999), and cattle (Pedernera et al., 2010), generally focusing on antioxidant supplementation and dietary trace mineral concentrations. Additionally, evaluation of dietary influence on OS in obese (Tanner et al., 2006) and renal insufficient (Yu and Paetau-Robinson, 2006) domestic cats revealed reduction of OS through high-protein diets in the obese cats and antioxidant (vitamins E and C and β-carotene) supplemented diets in renal insufficient cats. The influence of diet on OS in snow leopards has not been evaluated. Therefore, the objective of this study was to evaluate dietary nutrient intake and nutritional husbandry influence on markers of OS in male snow leopards maintained in U.S. zoos. It was hypothesized that dietary nutrients, particularly higher intakes of vitamins A and E, would reduce markers of OS and that trace mineral balance would also influence OS. This research has potential to improve diet formulations of exotic cats managed in zoos and to initiate future research evaluating diet and OS in relation to phenotypes and physiologies.

MATERIALS AND METHODS

All animal procedures were approved by each housing institutions' Animal Care and Use Committee (IACUC) before animal experimentation.

Animals

Fourteen male snow leopards (*Uncia uncia*) from 12 North American zoological institutions, accredited by the Association of Zoos and Aquariums, were used. None of the animals showed any clinical signs of disease or illness and none had documented diseases or illnesses. Detailed cat demographics, diet history, fasting days, and whole prey feeding frequency were surveyed (Table 1) for each animal at the time of sample collection through detailed interviews with animal managers. Animals ranged in age from 3 to 16 yr (average 8.5) and weight from 30.5 to 48.9 kg (average 37.9 kg; Table 2). All animals were fed primarily raw, commercially manufactured meat-based diets, formulated to meet cat nutrient requirements, with supplemental bones and/or whole prey items. Diets and amounts of diets were different for all animals except cats 8 and 9 that were fed identically. Animals were all managed at different institutions with exception of cats 7, 8, and 9 that were managed at the same zoo.

Sample Collection

Animals were immobilized after an overnight fast according to individual institution veterinary protocols by institution veterinarians using combinations of ketamine, medetomidine, butorphanol, and midazolam based on each institution's standard medical procedures. Approximately, 5 mL of fasted blood was collected via venipuncture of a jugular, saphenous, or femoral vein and was collected into 2 separate Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ),

Table 1. Relevant husbandry survey questions distributed to animal managers at each institution during snow leopard sample collections[†]

Q1.	What best describes the male's diet (if possible provide a sample copy of a weekly diet):
	a. Single commercial carnivore diet only.
	b. Variety or combination of commercial carnivore diets (include products and amounts)
	c. Commercial carnivore diet plus whole prey (rats, rabbits, birds, etc.).
	d. Whole prey items only
Q2.	If given whole prey items, what kind (type, size) and how often?
Q3.	Does this cat have a fast day? If so, how many days per week and what is offered?
Q4.	Using a body condition score index of 1 to 9, what is this cat's BCS?
Q5.	What is this cat's current body weight (include date the weight was obtained)?
Q6.	How often does the male receive enrichment items?
Q7.	Please provide examples of "preferred" enrichment items:

BCS = body condition score.

[†]Survey was given verbally by the same sample collector for each institution.

Table 2. Survey data demographics of male snow leopards housed in North American zoos

Cat	Weight, kg	Age, yr	Diet protein source(s)	Weekly as-fed intake, g	Weekly DMI, g	Fasting days†	Whole prey frequency‡
1	43.0	3.5	Beef	7,000	2,400	2	0.4
2	38.5	9.5	Horse	6,000	2,000	1	3.0
3	36.8	7.5	Horse	7,900	2,700	1	1.0
4	44.0	15	Horse	9,500	3,300	0	0.3
5	40.0	7	Pork	9,100	2,800	2	1.0
6	40.9	7	Beef, pork, and horse	6,600	2,100	1	1.0
7	32.3	6	Horse	10,900	3,600	1	1.0
8	30.5	3	Horse	8,200	2,700	1	1.0
9	30.5	11	Horse	8,200	2,700	1	1.0
10	41.0	11	Horse	4,800	1,700	3	3.0
11	41.8	7	Horse	5,400	1,700	1	0.3
12	30.7	5	Beef	6,000	2,100	0	0.5
13	48.9	16	Horse	6,400	1,900	0	2.0
14	32.2	11	Horse	7,000	2,400	2	0.5

†The number of days per week that animals were not fed commercially prepared raw meat diets.

‡Frequency was calculated by dividing total number of whole prey items offered per month (30 d) by 4 (wks).

one with no additive (blue top) for serum collection and the other one containing 1.8 mg ethylenediaminetetraacetic acid (EDTA)/mL (purple top) for plasma collection. Blood was processed at 24 °C according to institution protocol for routine blood diagnostics, centrifuged at approximately 1,000 × *g* for 10 min to separate serum and plasma, and stored at –80 °C until analyses. Approximately, 900 g of each animal's daily diet was obtained on the day of sample collection and stored at –18 °C until analyses. All collections took place between February 9 and June 14, 2016.

Diet Analyses

Approximately, 200 g of each diet sample was subsampled, dried at 55 °C, ground in a commercial blender (Waring blender model 51BL31, Waring, New Hartford, CT), and analyzed for chemical composition. All chemical analyses were conducted in the nutrition laboratory at Omaha's Henry Doorly Zoo and Aquarium unless otherwise noted and analyzed with a coefficient of variance less than 5%. Diets were analyzed for dry matter (DM; method 934.01) and organic matter (OM; method 942.05; AOAC, 2006). Crude protein (CP) was determined using a Leco Nitrogen/Protein Determinator (method 992.15; model TruMacN, Leco Corporation, St. Joseph, MI). Fat concentrations were determined by hexane extraction (method 960.39; AOAC, 2000). Total dietary fiber (TDF) was determined using the Prosky method (Prosky et al., 1994) adjusted for high-protein samples using quadruple the amount of protease and double the time for the water bath after the addition of protease.

Fatty acid analysis of raw meat diets was conducted at Iowa State University using gas chromatography (model 3800; Varian Analytical Instruments, Walnut Creek, CA) to determine fatty acid profiles (Richter et al., 2012). Peak identification and quantification (Kramer et al., 2008) were determined on esterified (Christie, 1972) lipid samples extracted from each diet sample (Folch et al., 1957). Mineral analyses of raw meat diets were conducted at Midwest Laboratories (Omaha, NE; method 985.01; AOAC, 2006). Diet subsamples from each institution were sent to Arizona State University for vitamins A (retinol) and E (α-tocopherol) analyses. Vitamins were analyzed via reverse-phase high pressure liquid chromatography (HPLC) as previously described (McGraw et al., 2006; Dierenfeld et al., 2009) using an Agilent 1100 Series (Santa Clara, CA) HPLC system.

Metabolizable energy (ME) concentrations of diets were estimated using Atwater values (9 kcal/g fat, 4 kcal/g protein, and 4 kcal/g carbohydrate) multiplied by fat, protein, and digestible carbohydrate content of each diet (NRC, 2006). Atwater values, opposed to modified Atwater values, have been suggested as more accurate for determinations of ME in raw meat diets because of high digestibility compared with processed and extruded diets (Clauss et al., 2010; Iske et al., 2016). Digestible carbohydrate concentrations were calculated using nitrogen-free extract (NFE) as an estimate with the following equation: [100 − (% ash + % CP + % fat + % TDF)]. Though crude fiber is typically used in this calculation, TDF is a more accurate measure of dietary fiber (Cho et al., 2001; de-Oliveira et al., 2012) and results in a more accurate estimation of

NFE. Due to the additive nature of the calculation and reliance on multiple assays along with very low carbohydrate and high protein and fat concentrations of the diets, calculated NFE of some treatments produced negative numbers, in which case a value of 0 was used.

Husbandry surveys concerning each cat's current age, weight, diet type, diet feeding amount, whole prey offered, whole prey feeding frequency, and number of fasting days were completed by housing institutions at the time of sampling via interview with animal keepers. Fasting days were defined as the number of days the animals were not fed their allotment of commercial meat diet only. Whole prey and enrichment items, such as bones, could be fed on those days. There is no recommended standard for fasting days, and zoos typically implement them for respective management considerations or provision of variety in normal routine of the animal, in an attempt to mimic the feeding cycle of wild cats. Although these types of fasting days do not mimic the gorge/fast feeding strategy of most large felids, they have been used extensively in zoos to provide variety (AZA Tiger Species Survival Plan®, 2016). To account for variation in fasting days, weekly dietary intakes were used and calculated by multiplying amount of diet fed on feeding days by the number of days cats were fed per week. Weekly intakes were then multiplied by DM concentration of the diet to give weekly dry matter intake (DMI). Analyzed dry matter nutrient concentrations were then multiplied by weekly DMI to yield weekly nutrient intakes on a dry matter basis (DMB) [((grams offered per day * d fed/wk) * %DM) * diet nutrient (%)]. Whole prey items, defined as nonliving whole animal carcass, as determined by survey responses included rabbit, rat, fish, guinea pig, chicken, and quail among all participating institutions. Whole prey items were not analyzed for chemical composition because they accounted for less than 10% of each animal's diet, based on the frequency of feeding whole prey and size of whole prey items. Therefore, nutrient intake was calculated based on commercial raw meat diet intakes only. Whole prey feeding frequency (d/wk provided) was assessed for correlations with OS.

Recommended weekly nutrient intakes were calculated based on published recommendations for domestic cats (NRC, 2006) as specific nutrient recommendations for snow leopards are not established. Calculations were made using published recommended allowance values (per 4,000 kcal ME/kg diet) adjusted to a 5,000 kcal ME/kg diet following dietary ME determination as described above. These values were then converted to gram basis that were then multiplied by 1,000, 2,000, 3,000, and 4,000 g of DMI to represent the recommendations across the various snow leopard weekly intakes.

Plasma samples also were sent to Arizona State University for vitamins A and E analyses via HPLC as described above (McGraw et al., 2006; Dierenfeld et al., 2009). Mineral analyses were conducted on blood serum samples at Iowa State University's Veterinary Diagnostic Laboratory via inductively coupled plasma mass spectrometry (ICP-MS, Analytik Jena Inc., Woburn, MA) with hydrogen gas. Briefly, samples were diluted 1:20 in 1% nitric acid, vortexed rigorously, and analyzed by ICP/MS with bismuth, scandium, indium, lithium, yttrium, and terbium used as internal standards.

Oxidative Stress Markers

Markers of OS were measured in plasma or serum. Analysis of OS markers included thiobarbituric acid reactive substances (TBARS), protein carbonyls (PC), DNA/RNA damage, SOD, and GPx activity using commercially available assay kits from Cayman Chemical Company (Ann Arbor, MI) and was performed according to the recommendations of the manufacturer (Table 3). Ferric reducing antioxidant power (FRAP), an overall total antioxidant capacity assay, was assessed in serum. Briefly, the FRAP assay colorimetrically measures the reduction of ferric iron (Fe^{+3}) to ferrous iron (Fe^{+2}) by the reaction of ferrous-tripyridyltriazine complex in relation to antioxidant-based ascorbic acid standards (Benzie and Strain, 1996). Samples did not require dilution and were expressed in units of μM (FRAP value). All assays were run in triplicate in a 96-well plate except for PC, which is analyzed in duplicate.

Statistical Methods

A total of approximately 80 explanatory variables were measured and evaluated in the current study, including individual dietary macronutrients, fatty acids, vitamin A, and mineral weekly intakes, as well as blood measures of OS, minerals, and vitamins A (retinol) and E. Explanatory variables were separated into categories (proximates, minerals, vitamins, fatty acids, and OS) and analyzed via multiple regression for their correlation with all response variables via the Regression procedure of SAS (SAS Inst. Inc., Cary, NC) with OS markers used as response variables. Due to large number of statistical regressions,

Table 3. Assay kits performed to determine OS in serum and plasma of male snow leopards[†]

Assay kit	Catalog number	Sample	Dilution factor	Standard curve reporting range	Biomolecular measures	OS indication	Reporting units
TBARS	700870	Serum	None	0 to 50 µM	Malondialdehyde	Lipid damage	µM
PC	10005020	Serum	None	N/A	2,4-dinetrophenylhydrazine	Protein damage	nmol/mL
DNA/RNA	589320	Plasma	1:74	10.3 to 3,000 pg/mL	8-hydroxy-2′-deoxyguanosine, 8-hydroxyguanosine, 8-hydroxyguanine	DNA or RNA damage	pg/mL
SOD[‡]	706002	Plasma	1:5	0.005 to 0.05 Unit/mL[‖]	Superoxide radical	Antioxidant	Unit/mL
GPx	703102	Plasma	1:20	0.5 to 1.2 initial absorbance	NADPH→NADP[+]	Antioxidant	nmol/min/mL

NADP = nicotinamide adenine dinucleotide phosphate; NADPH = nicotinamide adenine dinucleotide phosphate reduced.

[†]All kits were purchased from Cayman Chemical Company (Ann Arbor, MI).

[‡]Assay kit measures Cu/Zn, Fe, and Mn SOD.

[‖]One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

an attempt was made to highlight clinically significant variables by using a partial R^2 cutoff of 0.40 for significant variables. This was chosen to not only highlight variables with large influence but also assess many variables as this is the first research of this nature in snow leopards. Final statistical models included only significant variables. Age, body weight, frequency fed whole prey, and number of fasting days for each animal were also analyzed via regression analyses. A P-value of 0.05 was considered statistically significant.

RESULTS

Diet Intake

While exact nutritional management of cats varied, all snow leopards were fed raw, commercially manufactured beef, horse, or pork-based diets from Nebraska Brand (North Platte, NE), Triple A Brand Meat Company (Burlington, CO), Milliken Meat Products Ltd (Markham, Ontario), or Sustainable Swine Resources (Sheboygan Falls, WI). Protein sources of the commercial diets offered to cats included beef (n = 2), horse (n = 10), pork (n = 1), and combinations of all 3 protein sources (n = 1; Table 2). Leopards were managed with 0 to 3 fasting days each week and were offered 900 to 1,800 g as fed (300 to 600 g DMB) of meat on each non-fasting day. Whole prey offered ranged from once per month to 3 times/wk (Table 2) and accounted for less than 10% of the diet on average (by wet weight) due to very small prey items offered by all institutions (rabbit, rat, fish, guinea pig, chicken, or quail).

Nutrient concentrations of the commercial diets analyzed are presented in Tables 4 and 5. Initial analysis of vitamin E (α-tocopherol) concentrations was very low compared with expected values (1.0 ± 0.1 µg/g DMB). Therefore, some diets were analyzed at a secondary laboratory that resulted in different concentrations (107.8 ± 58.4 µg/g). Because of the limited sample size to reanalyze all diets and length of time the diets had been stored, vitamin E was left out of statistical analysis and is not presented. Average ME concentrations of the diets ranged from 4.5 to 5.9 kcal/g (DMB). Large variations in diet nutrient concentrations were measured (Tables 4 and 5), particularly in vitamin A (retinol) and copper, which ranged from 0.05 to 2.4 µg/g DM and 5.4 to 28.6 mg/kg DM, respectively.

Weekly DMI of commercial diets ranged from 1,256 to 3,551 g/wk with an average DMI

of 2,350 g (Table 6). Metabolizable energy intakes averaged 12,320 kcal/wk but ranged from 6,633 to 18,622 kcals/wk. The large range of DMI resulted in weekly nutrient intakes that also ranged widely, at least 2-fold, for every macronutrient (Table 6). On average, CP, fat, and TDF intakes represented 56.7%, 32.1%, and 5.4% of the DM, respectively. Average (± SD) fat (753.2 ± 264.0 g) and TDF (126.4 ± 41.3 g) intakes varied by 108% and 113%, respectively, among cats. Wide ranges in weekly vitamin A intakes were also observed (2,389 ± 1,983 µg). Weekly mineral intakes also had considerable ranges with the largest variations in intake measured for iron (1,321 ± 492.4 mg), copper (27.6 ± 19.2 mg), and manganese (90.1 ± 36.5 mg; Table 7).

Nutrient intakes of snow leopards were compared with calorically adjusted recommended nutrient allowances published for domestic cats. These comparisons indicated that consumption of protein and fat by snow leopards exceeded recommended allowances for all cats. Results also

Table 4. Macronutrient, ME, and retinol concentrations of commercial raw meat diets fed to male snow leopards (DMB)[†]

Cat	DM, %	OM, %	Protein, %	Fat, %	TDF, %	ME[‡], kcal/g	Retinol, µg/g
1	34.5	91.1	55.5	32.2	5.5	5.1	1.0
2	34.1	90.5	56.8	25.8	7.0	4.6	2.3
3	33.9	92.7	63.1	26.6	5.4	4.9	1.8
4	35.1	92.2	55.2	33.7	5.1	5.2	1.5
5	30.9	92.8	53.0	37.0	5.1	5.5	0.05
6	32.2	92.5	56.1	33.2	3.8	5.2	0.5
7	32.6	91.1	56.1	33.4	5.5	5.3	0.2
8	32.6	91.1	56.1	33.4	5.5	5.3	0.2
9	32.6	91.1	56.1	33.4	5.5	5.3	0.2
10	34.7	93.0	49.3	43.8	6.3	5.9	2.3
11	30.9	93.9	71.1	21.1	3.4	4.7	2.4
12	34.2	90.8	45.3	38.7	7.3	5.3	0.2
13	30.5	91.8	67.8	19.4	5.2	4.5	2.4
14	33.0	91.7	54.7	34.4	4.3	5.3	0.4
SD	1.6	1.0	7.2	7.2	1.2	0.4	1.0
Average	33.0	92.0	57.0	31.6	5.3	5.1	1.2

[†]Cats 7, 8, and 9 were fed the same diet.

[‡]Calculated using unmodified Atwater values: 9 kcal/g of fat + 4 kcal/g of CP + 4 kcal/g of NFE.

Table 5. Mineral composition of commercial raw meat diets fed to male snow leopards (DMB)[†]

Cat	S, %	P, %	K, %	Mg, %	Ca, %	Na, %	Fe, mg/kg	Mn, mg/kg	Cu, mg/kg	Zn, mg/kg	Zn:Cu
1	0.7	1.3	1.1	0.1	2.1	0.3	671.8	40.2	10.7	111.8	10.4
2	0.7	1.6	1.0	0.1	2.6	0.3	702.9	48.8	11.6	122.1	10.5
3	0.6	1.5	1.0	0.1	1.7	0.4	391.1	23.6	5.4	140.3	26.0
4	0.5	1.4	0.9	0.1	1.6	0.4	356.5	33.0	6.1	137.1	22.5
5	0.6	0.8	1.0	0.1	1.3	0.4	755.6	43.4	28.6	174.8	6.1
6	0.6	1.0	0.1	0.1	1.8	0.5	582.3	42.8	18.1	132.8	7.3
7	0.6	1.2	1.1	0.1	2.0	0.3	609.0	46.1	13.4	120.1	9.0
8	0.6	1.2	1.1	0.1	2.0	0.3	609.0	46.1	13.4	120.1	9.0
9	0.6	1.2	1.1	0.1	2.0	0.3	609.0	46.1	13.4	120.1	9.0
10	0.5	1.2	0.8	0.1	1.4	0.4	375.6	33.3	7.0	124.5	17.8
11	0.7	1.1	1.1	0.1	0.9	0.3	266.0	19.3	6.0	126.7	21.1
12	0.6	1.2	0.8	0.1	1.8	0.3	628.7	35.7	5.6	82.6	14.8
13	0.7	1.3	1.1	0.1	1.7	0.3	548.8	31.5	9.4	145.9	15.5
14	0.7	1.3	1.1	0.1	2.0	0.4	792.9	37.1	10.0	121.1	12.1
SD	0.1	0.2	0.3	0.0	0.4	0.1	158.9	8.9	6.2	20.3	6.2
Average	0.6	1.2	1.0	0.1	1.8	0.3	564.2	37.6	11.3	127.1	13.7

[†]Cats 7, 8, and 9 were fed the same diet.

Table 6. Weekly calculated macronutrient and vitamin intakes of male snow leopards from raw meat diets (DMB)[†,‡]

Cat	DMI, g	OM, g	Protein, g	Fat, g	TDF, g	ME[‖], kcals	Retinol, µg
1	2,400	2,209	1,346	779.3	132.3	13,997	2,320
2	2,000	1,845	1,158	526.9	143.8	9,440	4,713
3	2,700	2,476	1,686	710.3	144.5	13,139	4,724
4	3,300	3,078	1,843	1,125	170.3	17,499	5,043
5	2,800	2,605	1,489	1,040	142.0	15,313	126.9
6	2,100	1,974	1,197	708.0	81.4	11,160	991.1
7	3,600	3,233	1,991	1,185	194.7	18,622	845.1
8	2,700	2,425	1,493	888.5	146.0	13,967	633.9
9	2,700	2,425	1,493	888.5	146.0	13,967	633.9
10	1,700	1,553	823.9	731.5	105.8	9,878	3,783
11	1,700	1,580	1,197	354.5	56.4	9,296	4,072
12	2,100	1,875	935	799.4	151.5	10,935	396.9
13	1,900	1,780	1,314	375.9	101.0	8,639	4,683
14	2,400	1,151	687	431.8	53.9	6,633	485.7
SD	570.6	589.5	371.7	264.1	41.3	3,475	1,984
Average	2,436	2,158	1,332	753.2	126.4	12,320	2,389
Recommended Allowance/ 1,000 g DM[§]		—	250	112.5	—	—	1,250
Recommended Allowance/ 2,000 g DM		—	500	225.0	—	—	2,500
Recommended Allowance/ 3,000 g DM		—	750	337.5	—	—	3,750
Recommended Allowance/ 4,000 g DM		—	1,000	450.0	—	—	5,000

[†]Weekly macronutrient, energy, and vitamin intakes were calculated from individual raw meat diet analyses and diet records.

[‡]Cats 8 and 9 were fed the same amount of the same diet.

[‖]Calculated using unmodified Atwater values: 9 kcal/g of fat + 4 kcal/g of CP + 4 kcal/g of NFE.

[§]Recommended allowance for nutrients was calculated by adjusting the allowance column for adult cats for maintenance in the NRC (2006) to a diet containing 5,000 kcal ME/kg DM. These values are presented from 1,000 to 4,000 g of DM to represent the ranges of snow leopard intake.

indicated that all cats consumed at least twice the concentration of iron as recommended and on average consumed more than 5 times the recommended iron allowance. In contrast, 8 leopards failed to meet their adjusted vitamin A allowance based on their individual DMI indicating only 6 leopards consumed adequate concentrations of vitamin A for their individual respective DMI (Table 6). On average, potassium (23.4 g) and magnesium (2.3 g) intakes were 1.5 and 1.9 times greater than the adjusted average recommended allowances (15.3 and 1.2 g, respectively) based on the average DMI (2,400 g). Average intakes of phosphorus, calcium, sodium, iron, and manganese were 3.8 to 6.4 times more than adjusted average recommended allowances. Although average copper and zinc intakes both met or exceeded average adjusted allowances, 4 individuals (cats 3, 11, 12, and 14) did not meet the copper allowance and 2 individuals (cats 12 and 14) did not meet the zinc allowance when considering their individual DMI (Table 7).

Concentrations of plasma vitamins and minerals are presented in Table 8. Plasma vitamin concentrations varied widely between cats with differences of 71% and 112% measured for retinol (±SD; 4.3 ± 0.8 µg/mL) and vitamin E (11.2 ± 4.2 µg/mL), respectively. Plasma mineral concentrations varied from under 20% (calcium and magnesium) to above 60% for copper (0.06 ± 0.01 mg/dL) and iron (0.2 ± 0.1 mg/dL).

Oxidative Stress Markers

The activity of antioxidant enzymes (SOD and GPx) and antioxidant potential (FRAP) along with concentrations of DNA/RNA damage, TBARS, and PC as measured markers of OS in the blood are presented in Table 9. For SOD and DNA/RNA damage, assay values fell outside the range of the standard curve, which should be considered during interpretation as there was an inadequate sample to rerun assays. Average SOD and GPx activities were 1.9 ± 2.8 units/mL and

Table 7. Weekly calculated mineral intake of male snow leopards from raw meat diets (DMB)[†,‡]

Cat	DMI, g	S, g	P, g	K, g	Mg, g	Ca, g	Na, g	Fe, mg	Mn, mg	Cu, mg	Zn, mg	Zn:Cu
1	2,400	16.2	31.0	26.3	2.9	51.1	8.3	1,628	97.4	26.1	271.0	10.4
2	2,000	13.7	33.0	19.5	1.7	51.9	5.6	1,434	99.6	23.6	248.9	10.6
3	2,700	14.8	38.9	26.7	2.8	44.9	10.8	1,045	63.0	14.5	374.7	25.9
4	3,300	16.9	45.4	30.3	3.2	53.5	12.7	1,190	110.2	20.4	457.8	22.4
5	2,800	18.0	23.3	28.4	2.4	35.3	10.5	2,121	121.8	80.2	490.6	6.1
6	2,100	13.4	20.6	19.5	2.0	38.8	10.4	1,243	91.4	38.5	283.4	7.4
7	3,600	21.7	43.8	38.0	3.9	71.7	12.0	2,162	163.5	47.7	426.3	8.9
8	2,700	16.3	32.8	28.5	2.9	53.8	9.0	1,622	122.7	35.8	319.7	8.9
9	2,700	16.3	32.8	28.5	2.9	53.8	9.0	1,622	122.7	35.8	319.7	8.9
10	1,700	7.7	20.6	13.5	1.5	24.1	6.7	627.0	55.6	11.7	207.8	17.8
11	1,700	11.0	18.8	18.3	1.6	15.3	5.3	447.6	32.5	10.1	213.1	21.1
12	2,100	11.4	25.1	15.6	1.3	36.4	5.4	1,298	73.7	11.6	170.5	14.7
13	1,900	13.6	25.3	20.4	1.6	32.8	4.9	1,064	61.0	18.3	282.8	15.4
14	2,400	8.7	15.9	14.0	1.4	25.1	4.4	995.8	46.6	12.5	152.2	12.2
SD	570.6	3.8	9.3	9.4	0.8	15.1	2.8	492.4	36.5	19.2	104.6	6.1
Average	2,436	14.2	29.0	23.4	2.3	42.0	8.2	1,321	90.1	27.6	301.3	13.6
Recommended Allowance/1,000 g DM[‖]			3.3	6.5	0.5	3.6	0.9	100.0	6.0	6.3	92.5	
Recommended Allowance/2,000 g DM			6.6	13.0	1.0	7.2	1.8	200.0	12.0	12.6	185.0	
Recommended Allowance/3,000 g DM			9.9	19.5	1.5	10.8	2.7	300.0	18.0	18.9	277.5	
Recommended Allowance/4,000 g DM			13.2	26.0	2.0	14.4	3.6	400.0	24.0	25.2	370.0	

[†]Weekly mineral intakes were calculated from individual raw meat diet analyses and diet records.

[‡]Cats 8 and 9 were fed the same amount of the same diet.

[‖]Recommended allowance for nutrients was calculated by adjusting the allowance column for adult cats for maintenance in the NRC (2006) to a diet containing 5,000 kcal ME/kg DM. These values are presented from 1,000 to 4,000 g of DM to represent the ranges of snow leopard intake.

Table 8. Concentrations of plasma vitamins and serum minerals in male snow leopards[†]

Cat	Retinol, µg/mL	α-Tocopherol, µg/mL	Ca, mg/dL	Cu, mg/dL	Fe, mg/dL	K, mg/dL	Mg, mg/dL	P, mg/dL	Zn, mg/dL
1	3.4	12.7	9.0	0.05	0.1	14.6	2.0	6.3	0.05
2	3.9	9.4	7.9	0.05	0.1	17.4	1.9	5.6	0.05
3	5.1	6.6	8.2	0.04	0.2	14.7	1.9	4.4	0.04
4	4.4	19.4	7.9	0.05	0.1	16.5	2.0	5.2	0.04
5	4.0	7.8	8.8	0.06	0.1	17.1	2.0	5.6	0.05
6	4.6	10.0	8.0	0.05	0.4	14.9	1.8	5.6	0.06
7	3.7	7.0	8.5	0.07	0.6	17.4	2.0	5.1	0.06
8	4.2	5.5	8.8	0.07	0.2	17.7	2.0	5.0	0.05
9	4.5	8.7	8.3	0.06	0.1	13.2	1.9	5.4	0.05
10	4.1	14.6	9.0	0.08	0.1	16.2	1.9	5.8	0.05
11	5.9	10.9	7.5	0.04	0.1	13.7	2.1	4.4	0.07
12	5.1	15.7	8.9	0.07	0.3	17.6	2.0	5.9	0.05
13	2.8	17.2	8.3	0.08	0.1	17.8	2.0	7.0	0.05
14	4.3	11.2	—	—	—	—	—	—	—
SD	0.8	4.2	0.5	0.01	0.1	1.6	0.1	0.7	0.01
Average	4.3	11.2	8.4	0.06	0.2	16.1	2.0	5.5	0.05

[†]Due to the small sample size, plasma minerals could not be analyzed for cat 14.

2,088 ± 1,609 nmol/min/mL, respectively. Analysis of blood GPx activity in 4 cats resulted in negative values; therefore, they were not included in average calculations or statistical analysis. Antioxidant potential (FRAP) averaged 255.6 ± 59.5 µM. Markers of OS damage including DNA/RNA damage, TBARS, and PC resulted in average values of 5,414 ± 1,232 pg/mL, 13.8 ± 2.1 µM, and 24.4 ± 10.1 nmol/mL, respectively. Intra- and inter-assay coefficient of variations for SOD, GPx, FRAP, DNA/RNA damage, TBARS, and PC were 8.6% and 98.8%, 3.4% and 73.5%, 7.0% and 23.3%, 8.1% and 22.7%, 4.3% and 15.3%, and 8.1% and 41.5%, respectively.

Significant correlations with OS markers are presented in Table 10 and Fig. 1. Dietary intakes of copper and sodium were the only nutrients correlated with markers of OS. Dietary copper intake was negatively correlated (P = 0.01) with blood DNA/RNA damage (R^2 = 0.44) indicating that an elevated intake of copper was associated with less DNA/RNA damage. Additionally, elevated GPx activity (R^2 = 0.50) was positively correlated (P = 0.04) with higher intakes of sodium.

Whole prey items were offered to snow leopards 0.3 to 3 times/wk (Table 2). More frequent feeding of whole prey resulted in a positive correlation (P < 0.01) with SOD activity (R^2 = 0.55) in the blood (Table 10 and Fig. 1B).

DISCUSSION

The influence of various nutrients on OS has been well documented in many domestic species but has not been effectively evaluated in exotic species. Comparison of domestic and exotic animals leaves room for error due to unique diets and physiologies; however, these comparisons provide an initial baseline for extrapolation. Domestic cats are typically used as an adequate model for exotic felids; however, lack of established nutrient requirements specifically for exotic species leads to challenges in diet formulations that could result in imbalances of certain nutrients, possibly promoting OS. Results of studies assessing OS can be difficult to interpret as physiological responses to OS vary depending on severity, which itself is ill-defined as "baseline" or "normal" levels of OS are unknown and likely to vary by species. For example, antioxidant enzyme activity may actually increase in states of "mild" OS as an adaptation to protect the cell (Halliwell and Gutteridge, 2015). Additionally, many of these studies utilize artificial methods to induce nutrient overloads. Understanding and assessment of OS in relation to nutrient intake could be extremely useful for animal management as many diseases and physiologies, including renal insufficiency (Yu and Paetau-Robinson, 2006) and sperm quality (Thuwanut et al., 2011), can be influenced by damage to proteins, lipids, and DNA

Table 9. Concentrations of markers of OS in blood of male snow leopards

Cat	SOD[†,‡], U/mL	GPx[‖], nmol/min/mL	FRAP, µM	DNA/RNA Damage[§], pg/mL	TBARS, µM	PC, nmol/mL
1	1.7	1,527	242.0	3,265	13.4	14.1
2	10.7	–	177.2	6,316	15.1	26.5
3	0.7	4,422	254.9	6,038	13.9	20.3
4	1.9	4,167	252.1	5,263	11.7	26.7
5	1.8	26.2	225.7	5,627	16.1	34.0
6	0.4	2,654	284.0	4,710	14.2	26.3
7	0.03	–	183.5	3,748	14.5	23.9
8	0.06	3,339	321.7	5,221	15.9	37.8
9	0.00	1,909	187.6	4,141	13.6	18.5
10	5.0	1,559	275.1	4,484	14.4	14.7
11	0.8	1,111	236.7	6,985	9.0	0.8
12	1.4	–	405.7	7,347	12.0	33.3
13	1.3	164.5	279.2	6,618	17.1	27.7
14	1.0	–	252.8	6,028	12.1	36.4
SD	2.8	1,609	59.5	1,232	2.1	10.1
Average	1.9	2,088	255.6	5,414	13.8	24.4

[†]One unit (U) of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

[‡]Assay values for cats 1 to 6 and 10 to 14 fell outside the range of the standard curve.

[‖]Samples with "–" had negative values for GPx analysis.

[§]Assay values for all cats fell outside the range of the standard curve.

as a result of OS. The objective of this study was to evaluate the nutrient influence and nutritional husbandry on OS markers in male snow leopards maintained in U.S. zoos. While significant correlations do not indicate cause-and-effect relationships, they can indicate the influence or impact of variables on one another and be applied to dietary formulations. Additionally, they can be further evaluated in relation to phenotypes, inclusive of presence or absence of clinical disease or illness or reproductive characteristics. Baseline data are critically important to develop additional research questions that fill knowledge gaps for species and allow for improvements in animal management.

Table 10. Significant variables from multiple regression analyses of diet proximates, minerals, vitamins, and fatty acids on markers of OS in the blood of male snow leopards

Marker	Variable	R^2	Correlation	*P*-value
Oxidative damage markers				
DNA/RNA Damage	Copper	0.44	Negative	0.01
Antioxidant markers				
SOD	Whole prey frequency	0.55	Positive	<0.01
GPx	Sodium	0.43	Positive	0.04

Diet Intake

All macronutrient intakes exceeded the calorically adjusted nutrient allowances for domestic cats. Crude protein and fat intakes represented on average 56.7% and 32.1% of the DM, respectively, while TDF intakes represented 3.4% to 7.3% of the DM and averaged 5.4%. The variation in DMI resulted in a range of ME intakes from 6,633 to 17,499 kcals/wk (948 to 2,493 kcal/d). Daily ME requirements for adult exotic cats (NRC, 2006) are estimated at 55 to 260 kcal × kg $BW^{0.75}$. When considering individual metabolic body weights, snow leopards in the current study consumed an ME range of 70.1 to 196.4 kcal × kg $BW^{0.75}$ with an average of 117.3 kcal × kg $BW^{0.75}$. This was within the range of estimated exotic cat requirements presented in the 2006 National Research Council (NRC) and provides a narrower range of estimated daily energy which may be particularly useful to snow leopard managers.

Dietary vitamin A intakes compared with adjusted recommendations suggested that 8 of the snow leopards did not consume adequate amounts of vitamin A, though this does not factor in whole prey consumption that would provide additional vitamins to the diet. This was a result of the less than expected measured concentrations of retinol in the respective diets. Commercial diets are

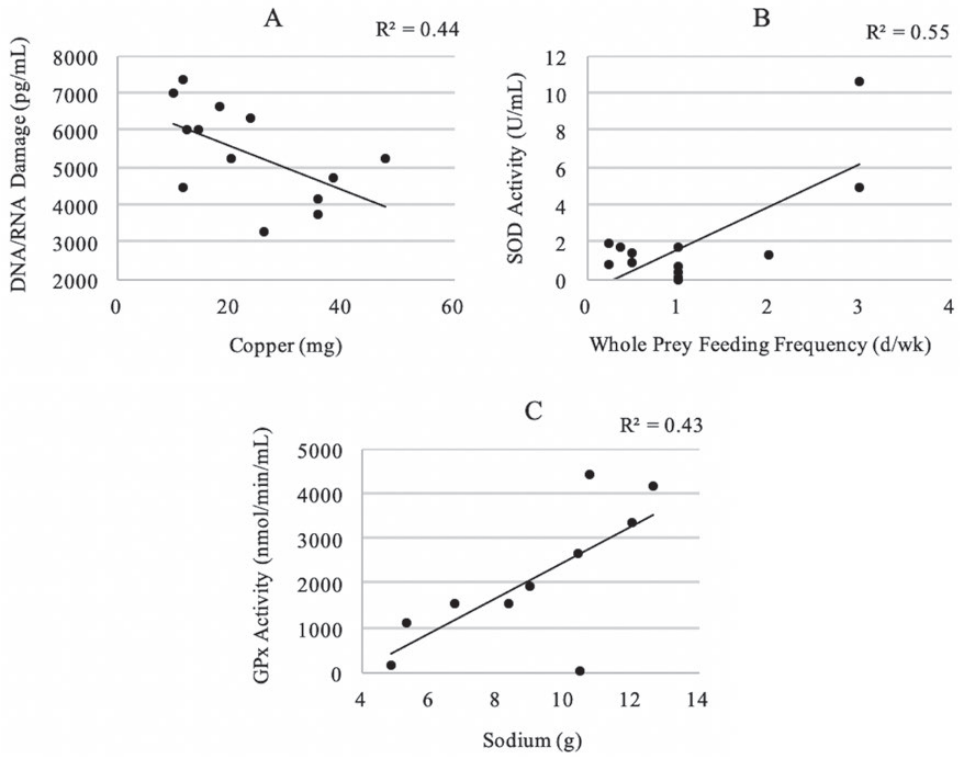

Figure 1. Linear regression of (A) copper ($P = 0.01$), (B) whole prey ($P < 0.01$), and (C) sodium ($P = 0.04$) intakes with markers of OS in the plasma of male snow leopards.

formulated to contain vitamin concentrations that meet or exceed domestic cat recommended allowances. For example, Nebraska Brand (Nebraska Packing Inc., North Platte, NE) approximates vitamin A concentrations of their feline diets to be between 3.1 and 3.4 µg/g (DM); however, measured concentrations in the current study only ranged from 0.11 to 1.4 µg/g (DM).

The less than expected intakes of vitamin A may be partially explained by protein source (Lee et al., 2007; Wood et al., 2008). The majority of snow leopards (n = 10) consumed horse as the primary protein source compared with beef (n = 2), pork (n = 1), or a combination of all 3 protein sources (n = 1). On average, cats consuming the horsemeat-based diets consumed 2.4 and 5.3 times more vitamin A (2,962 µg DM) compared with those fed beef or pork (1,236 and 559.0 µg, respectively). Unfortunately, because 10 of the 14 animals were fed horse-based diets and only 1 animal fed pork, conclusions about the protein source cannot be made. However, it is interesting to note that differences in carotenoid concentrations of grass-fed compared with grain-fed ruminants have been documented and suggest that diet of the animal prior to harvest plays a critical role in the nutrient composition of the meat (Daley et al., 2010). It is possible that horses fed more forage compared with beef and pork counterparts produce meat that contains naturally higher concentrations of vitamin A (Daley et al., 2010). It would be interesting to measure the vitamin concentrations of the source meats prior to commercial production and supplementation with premix formulations.

Another possible explanation for less than expected vitamin A concentrations in the snow leopard diets was storage conditions and diet sample handling. Storage and freezing conditions (Desai, 1984) of commercial raw meat diets in zoos need careful consideration and evaluation as they likely vary drastically among institutions. From the date of manufacture, diet samples were frozen between 30 and 365 d. The freezing process can be detrimental to fat-soluble vitamins largely due to lipid and fatty acid concentrations in the diet. For example, vitamin A (retinol) concentrations in chicken livers were more than 44% less than fresh livers after 90 d of freezing at −18 ° C (Dos Santos et al., 2009). Fat can breakdown to form hydroperoxides, which are relatively stable at low temperatures, and can oxidize vitamins (Bender, 1992). The thawing process of frozen meats and storage temperature fluctuations are also important as continued fat degradation can form more hydroperoxides to further oxidize fat-soluble vitamins. Additionally, less investigated theories could be causing vitamin disappearance, such as microbial vitamin utilization or destruction. Raw meat diets contain high concentrations of a variety of microorganisms (Hellgren et al., 2019). Understanding the interaction of bacterial species in raw meat along with resulting vitamin concentrations could be useful to diet formulations that use raw meat as the primary ingredient of diets.

Laboratory error and/or variation obviously occurred in analysis of vitamin E. A variety of methods exist for vitamin E determination in foods that include human error. Because of this, the HPLC method has been recommended for animal tissue and was the methodology utilized by both analyzing labs in the current study (Desai, 1984). Even using the same methodology, error can still occur and inter-laboratory variation exists. For this reason, it is important to evaluate results with expected values and validate laboratory testing methods.

Plasma vitamin E concentrations in the current study (5.5 to 19.4 µg/mL) were in range with serum vitamin E concentrations (5.6 to 16.8 µg/mL) reported in leopards (Ghebremeskel and Williams, 1988; Crissey et al., 2003). Similarly, plasma retinol concentrations in the current study (2.8 to 5.9 µg/mL) were greater than plasma and serum concentrations previously reported for leopards (*Panthera pardus*, *Uncia uncia*; 0.4 to 0.6 µg/mL) (Ghebremeskel and Williams, 1988; Crissey et al., 2003) even in the cats that consumed less retinol than recommended. This could support the theory that laboratory evaluations of the diets were indeed measured consistently low. Additionally, plasma analysis of fat-soluble vitamins is not an ideal method to evaluate status. Absorption and metabolism of key nutrients are highly regulated, and plasma concentrations may only change in cases of extreme deficiency or toxicity (Gropper and Smith, 2013). Measurement of vitamins in storage organs such as the liver would be the more accurate measurement locations for vitamin status; however, it is difficult to obtain (Albahrani and Greaves, 2016).

Maintenance of plasma mineral concentrations was also evident in our analysis. Average copper intake was 27.6 mg compared with the average adjusted allowance of 14.7 mg; however, 4 snow leopards consumed less than the

recommended amount for their respective DMI. Even though the copper intakes varied by nearly 160% and some intakes were low, plasma copper concentrations only varied by 67% (average 0.06 mg/dL) and fell into ranges previously reported for domestic cats and cheetahs (0.02 to 0.1 mg/dL; Dierenfeld, 1993; Vester et al., 2010; Depauw et al., 2012). Average plasma calcium (8.4), iron (0.2), magnesium (2.0), phosphorus (5.5), zinc (0.05), and potassium (16.1) concentrations (mg/dL) also fell into ranges previously reported for exotic and domestic cats (5.6 to 11.6, 0.02 to 0.1, 0.06 to 0.2, 1.9 to 2.4, 1.5 to 6.5, 0.06 to 0.2, and 3.6 to 21.5 mg/dL; Dierenfeld, 1993; Vester et al., 2010; Depauw et al., 2012). Though cats consumed 2.6 to 7.6 times the recommended iron allowance, plasma iron concentrations fell into expected ranges. However, concentration and balance of minerals should be carefully evaluated to avoid toxicity or long-term consequences of increased mineral load.

Oxidative Stress Markers

Measures of plasma OS vary largely between species (Maral et al., 1977; Marklund et al., 1982; Vernet et al., 2004) with differences of nearly 100-fold being reported across laboratory and livestock animals. The activity of plasma SOD in snow leopards (average 1.9 U/mL) was lower than observed in domestic cats (*Felis catus*; 9.6 U/mL; Marklund, 1984). It is interesting to note that the 3 cats housed in the same institution had the lowest SOD activity, which could indicate an impact of diet or husbandry on this marker, though consistent differences were not observed for these 3 cats in any other markers. Overall, cats have lower SOD activity compared with rats (339.1 U/mL) and humans (29.7 U/mL; Marklund, 1984). Plasma GPx activity and lipid damage in healthy domestic cats averaged 6.7 nmol/min/mg protein (334 to 668 nmol/min/mL assuming 50 to 100 mg protein per mL plasma; Piyarungsri and Pusoonthornthum, 2016) and 2.3 µM (Todorova et al., 2005), respectively, in previous studies, which are considerably less than our measured values (2,088 nmol/min/mL and 13.8 µM). Some OS markers such as FRAP, PC, and DNA/RNA damage have not been evaluated in domestic cats, and thus, other models must be used for comparison. Concentrations of plasma FRAP (255.6 µM) and protein damage (24.4 nmol/mL) in snow leopards fell outside ranges documented in human plasma (400 to 1,000 µM; Lotito and Frei, 2004, 2006) and 9.8 to 19.0 nmol/mL (Rossner et al., 2007; Yeh et al., 2008), respectively. Plasma DNA/RNA damage found in the current study (5,414 pg/mL) was within ranges found in rats (430 to 6,630 pg/mL; Kadiiska et al., 2005). Discrepancies observed in plasma OS markers between snow leopards, domestic cats, and other species are likely associated with species differences that make comparisons and conclusive inferences difficult. If wide differences between species exist, a dataset of values for an unevaluated species will be valuable for future comparisons.

Of all the nutrients evaluated, only copper and sodium intakes were correlated with markers of OS, with elevated copper intakes correlated with reduced DNA/RNA damage and elevated sodium intakes correlated with greater GPx activity. The role of dietary copper in OS is not clear as both copper deficiency and toxicity can lead to OS (Gaetke and Chow, 2003). Copper supplementation (175 mg/kg) well-above requirements (10 mg/kg) in the diet of growing pigs resulted in a 9.0% reduction in plasma lipid damage compared with control diets with no supplemental copper, suggesting an antioxidant role of copper, though it had no significant effect on SOD or GPx (Lauridsen et al., 1999). Reductions in plasma DNA/RNA damage observed in the current study with higher copper intakes may result from reduced superoxide radical formation via the catalytic role of copper in SOD (Halliwell and Gutteridge, 2015). Though this was not directly observed in the current study, it deserves further investigation. Based on the average intakes and adjusted nutrient allowances from the NRC, the ideal zinc to copper ratio for cats was 14.7, while the actual average intake ratio in the current study for snow leopards was 13.6. With the evidence that both copper and zinc act as oxidants and antioxidants and are antagonists, this ratio is likely very important when considering diet formulations. The ratio has been studied in relation to age-related degenerative diseases and OS in humans; therefore, the results of the present study indicate that it likely has importance for snow leopards (Mezzetti et al., 1998; Malavolta et al., 2015). Any future research with cats related to dietary copper should also include consideration of zinc.

Average sodium intakes were 4 times above recommended allowances and intakes were well above adequate in all leopards, with higher intakes correlated with increased GPx activity. Little work has been done assessing the correlation between dietary sodium and OS. High sodium diets have resulted in

increased OS in rats (Hummel et al., 2012); therefore, this should be investigated in cats to assess the implications of the observed heightened GPx activity. Caution should be taken, however, to avoid concentrations of dietary sodium that may have undesired effects, as leopards were already consuming more than the recommended allowance in the current study. This may be particularly important to consider when working with geriatric, hypertensive, or renal insufficient animals (Elliott, 2006; Jepson et al., 2007).

Zoos often provide whole prey items as dietary enrichment. In the current study, all leopards were offered whole prey, but frequency ranged from 3 times/wk to once per month (0.3 times/wk). Consumption of whole prey provides a variety of nutrients that may impact metabolism, including insoluble animal fiber (Depauw et al., 2013). Alterations in metabolism may provide some explanation of the correlation between increased frequency of feeding of whole prey and increased SOD activity. The addition of soluble fiber (apple pectin, cocoa fiber, or β-glucan at 5% or 10% of the diet) to rat diets resulted in approximately 70% less plasma lipid peroxidation (Sánchez et al., 2011), while fermentable fiber (source not named) supplementation (10 and 20 g/d) significantly increased total antioxidant capacity (Xie et al., 2015) compared with diets with no added fiber. Conversely, other studies using rats found little to no differences in lipid peroxidation between soluble (barley) and insoluble (cellulose and wheat bran) dietary fiber types (Belobrajdic et al., 2011). Conclusions on the role of dietary fiber in OS have not always been agreed (Saha et al., 2017) and have not been evaluated in hypercarnivores such as cats. There were no correlations with markers of OS and TDF intakes that ranged from 3.4% to 7.3% of DM. Further research evaluating the role of whole prey and animal fiber is warranted as results indicate some markers of OS may be impacted by whole prey. Furthermore, future research should focus on differentiation and analysis of whole prey types, along with specific nutrient intakes as well as physiological impact from consumption of whole prey.

Conclusions

The current study is among the first to assess the correlation between nutrient intake and OS in an exotic cat species, thus providing additional data that may advance diet formulations. It was hypothesized that higher vitamins A and E intakes would reduce markers of OS and trace mineral balance would influence OS. In partial support of our hypothesis, sodium and copper intakes influenced GPx activity and DNA/RNA damage in male snow leopards, respectively. Very high intakes of iron indicate a need to carefully evaluate mineral concentrations and mineral balance in zoo carnivore diet formulations. While higher concentrations may be warranted for growth and reproduction, animals at maintenance likely do not require the very high concentrations of minerals measured in the current study. Contrary to our hypothesis, vitamin intake did not directly impact markers of OS. However, a more extensive study is required to address the stability of fat-soluble vitamins in raw meat diets including storage and handling to prevent potential degradation and loss. Based on the very low estimated intakes observed in the present study, supplementation of vitamin A may be warranted and should be discussed within management teams to ensure adequate yet safe intakes, although replication of analysis in additional laboratories should also be considered. Based on markers of OS that we assessed, provision of weekly whole prey supplementation and higher dietary copper and sodium intakes may mitigate OS. Replication of this work should be expanded to other cat species to validate findings and assess species differences in markers of OS.

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