EVALUATION OF DIET INFLUENCE ON OXIDATIVE STRESS AND ITS IMPACT ON SEMEN QUALITY IN SNOW LEOPARDS (*UNCIA UNCIA*)

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Abstract

The snow leopard (*Uncia uncia*) population in zoos has been declining over the past two decades and since 2008 less than 30% of recommended breeding pairs have produced litters. Snow leopards are listed as an endangered species by the International Union for Conservation of Nature and Natural Resources and improved reproductive success is vital for species conservation. Reduced sperm quality is a possible factor influencing reproduction and may be caused partly by oxidative stress (OS). Diet influences oxidative status and sperm are prone to oxidative damage because of high levels of polyunsaturated fatty acids. The objective of this study was to evaluate dietary influence on OS and its influence on sperm quality in snow leopards maintained in U.S. zoos.

Diet samples, blood, sperm (n=14), and seminal fluid (n=9) were collected from male snow leopards (average age 8.5, average weight 37.9 kg) between February and June 2016. Diet samples were analyzed to determine concentrations of macronutrients, energy, vitamin A (retinol, retinol ester, β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin) and E (α-tocopherol), minerals, and fatty acids; blood and seminal fluid were analyzed for OS markers: protein carbonyls, thiobarbituric acid reactive substances (TBARS), DNA/RNA damage, superoxide dismutase (SOD), glutathione peroxidase (GPx), and ferric reducing antioxidant potential (FRAP), as well as vitamin A and E, and minerals; ejaculates were evaluated for total sperm count, normal morphology, and motility. Regression analysis was used to evaluate relationships between weekly nutrient intakes, OS markers (Tables 1 and 2), and sperm quality (Table 3).

Total sperm count and percent normal morphology were significantly ($P<0.05$) and positively correlated ($R^2=0.81$). Iron intake had a significant ($P<0.05$) positive relationship with protein carbonyls ($R^2=0.47$) and TBARS ($R^2=0.33$) in blood and a significant negative relationship with DNA/RNA damage ($R^2=0.68$), SOD activity ($R^2=0.47$), and FRAP ($R^2=0.60$) in seminal fluid. Copper intake had a significant ($P<0.05$) negative relationship with SOD activity ($R^2=0.76$) and DNA/RNA damage ($R^2=0.57$) seminal fluid, and tended ($P<0.10$) to have a negative relationship with FRAP in seminal fluid ($R^2=0.41$) and a positive relationship with TBARS in blood ($R^2=0.23$). Zinc intake directly impacted OS markers along with its intake ratios with iron and copper. Zinc intake had a significant ($P<0.05$) negative relationship with DNA/RNA damage ($R^2=0.65$), SOD activity ($R^2=0.58$), and FRAP ($R^2=0.44$) in seminal fluid. Zn:Cu had a significant ($P<0.05$) positive relationship with SOD activity in seminal fluid ($R^2=0.48$) and tended ($P<0.10$) to have a positive relationship with DNA/RNA damage ($R^2=0.43$) and FRAP ($R^2=0.34$) in seminal fluid and a negative relationship with TBARS in blood ($R^2=0.22$). Fe:Zn had a significant positive relationship with protein carbonyls in blood ($R^2=0.28$). Sulfur had a significant negative relationship with DNA/RNA damage ($R^2=0.77$),

SOD activity (R^2=0.60), and FRAP (R^2=0.52) in seminal fluid. Calcium intake and ratio with phosphorus had impacts on OS damage. Calcium intake had a significant negative relationship with DNA/RNA damage (R^2=0.65), SOD activity (R^2=0.50), and FRAP (R^2=0.60) in seminal fluid and tended to have a positive relationship with GPx activity (R^2=0.37) and protein carbonyls (R^2=0.22) in blood. Ca:P had a significant positive relationship with protein carbonyls in blood (R^2=0.30) and tended to have a negative relationship with DNA/RNA damage (R^2=0.22) and a positive relationship with TBARS (R^2=0.23) in blood. Ca:P also tended to have a negative relationship with SOD activity in seminal fluid (R^2=0.34). Dietary α-tocopherol intake had a significant negative relationship with DNA/RNA damage (R^2=0.62), SOD activity (R^2=0.54), and FRAP (R^2=0.73) in seminal fluid and tended to have a positive relationship with TBARS in blood. Beta-carotene intake had a significant positive relationship (R^2=0.39) and α-carotene tended also to have a positive relationship (R^2=0.36) with GPx activity in blood. Retinol ester consumption had a significant positive relationship (R^2=0.32) and retinol consumption tended also to have a positive relationship (R^2=0.20) with SOD activity in blood.

Average total sperm count was 214.3 million sperm per ejaculate and average percent normal morphology was 27.3%. Dietary intakes were calculated weekly to account for different number of fasting days between cats. Weekly intakes of dry matter (2274.8 ± 186.2 g), organic matter (2089.0 ± 170.3 g), crude protein (1290.5 ± 107.4 g), total fat (728.9 ± 74.0 g), total dietary fiber (122.3 ± 11.6 g) and metabolizable energy (11,727.1 ± 1014.4 kcals) were not correlated ($P>0.05$) with total sperm count or morphology. Total sperm count had a positive relationship ($P<0.05$) with dietary intake of lauric acid (R^2=0.72), omega-3 (R^2=0.53), alpha-linolenic (R^2=0.52), omega-3:omega-6 (R^2=0.47), and docosenoic (R^2=0.41) fatty acids as well as dietary phosphorus (R^2=0.33) and retinol ester (R^2=0.32) intake and GPx activity (R^2=0.61). Dietary lignoceric acid (R^2=0.33) and serum copper (R^2=0.36) and phosphorus (R^2=0.30) levels were negatively correlated with total sperm count ($P<0.05$). The proportion of spermatozoa with normal morphology was positively correlated ($P<0.05$) with dietary copper (R^2=0.63), iron (R^2=0.42), manganese (R^2=0.36), and sulfur (R^2=0.34, P=0.05) intakes. Beta-cryptoxanthin (R^2=0.41), zinc:copper (R^2=0.38), retinol ester (R^2=0.37), α-tocopherol (R^2=0.37), and β-carotene (R^2=0.33) intakes had a negative relationship with normal sperm morphology. Activity of SOD (R^2=0.90) and protein carbonyl content (R^2=0.76) in seminal fluid were both negatively correlated ($P<0.05$) with sperm morphology.

Age, body weight, frequency fed whole prey, and number of fasting days showed no significant relationships with sperm count or morphology, so variables were grouped for further analysis: frequency that cats were fed whole prey (less than once per week, once per week, more than once per week), age (3-6, 7-10, and 11-16 years), and body weight (30-33, 35-41, and 42-49 kg). There were no significant differences in total sperm count between cats fed whole prey less than once per week, one time per week, or more than once per week, however, cats fed whole prey once per week had higher ($P <0.05$) percent normal morphology than cats fed whole prey less than once per week. There also were no significant differences in total sperm count and percent normal morphology between cats fed a single type of commercial diet or a combination of multiple commercial diets. Animals in the middle age and body weight ranges (7-10 years old and 35-41 kg) tended (P = 0.06, 0.08) to have higher quality sperm (total sperm count and morphology, respectively) than older and heavier cats.

In conclusion, fatty acid, trace mineral, and vitamin A and E intakes may influence sperm quality. Lauric acid, omega-3, linolenic acid, and docosenoic fatty acids specifically appeared to improve sperm production. Iron and copper intake appear to increase oxidative damage markers in blood and reduce antioxidants in seminal plasma, but increase the percent of normal sperm. This may indicate an alternative mechanism of iron and copper influence on sperm quality. Vitamin A and E also appear to have differing effects on OS and sperm quality. In general, dietary vitamin E reduced antioxidants in seminal fluid but improved sperm morphology while vitamin A increased antioxidants in blood and increased sperm count but reduced normal sperm morphology. Vitamins and trace minerals should be further evaluated to determine mechanisms and ideal dietary inclusions and ratios. Maintaining cats at a body weight between 35 and 41 kg and feeding whole prey at least once a week may also have potential to improve sperm quality.

Table 1. Regression analyses of weekly dietary intakes with oxidative stress damage markers.

Measure	Sample	Nutrient	R^2	P-Value	Association
DNA/RNA	Blood	Ca:P	0.22	0.09	Negative
	Seminal Fluid	S	0.77	0.002	Negative
		Fe	0.68	0.006	Negative
		K	0.68	0.007	Negative
		CP	0.66	0.008	Negative
		Ca	0.65	0.008	Negative
		Zn	0.65	0.009	Negative
		α-tocopherol	0.62	0.01	Negative
		P	0.59	0.02	Negative
		Cu	0.57	0.02	Negative
		DM	0.57	0.02	Negative
		Mn	0.57	0.02	Negative
		Mg	0.56	0.02	Negative
		OM	0.56	0.02	Negative
		ME	0.43	0.06	Negative
		Zn:Cu	0.43	0.06	Positive
		TDF	0.34	0.10	Negative
Protein Carbonyls	Blood	Fe	0.47	0.007	Positive
		Ca:P	0.30	0.04	Positive
		Fe:Zn	0.28	0.05	Positive
		Mn	0.27	0.06	Positive
		Ca	0.22	0.09	Positive
	Seminal Fluid	---			
TBARS	Blood	Fe	0.33	0.03	Positive
		Ca:P	0.23	0.08	Positive
		Cu	0.23	0.10	Positive
		Mn	0.22	0.09	Positive
		α-tocopherol	0.22	0.09	Positive
		Zn:Cu	0.22	0.09	Negative
	Seminal Fluid	---			

Table 2. Regression analyses of weekly dietary intakes with antioxidant enzymes and capacity.

Measure	Sample	Nutrient	R^2	P-Value	Association
SOD	Blood	Retinol ester	0.32	0.03	Positive
	Blood	Retinol	0.20	0.10	Positive
	Seminal Fluid	Cu	0.76	0.002	Negative
		S	0.60	0.01	Negative
		Zn	0.58	0.02	Negative
		OM	0.57	0.02	Negative
		DM	0.56	0.02	Negative
		Mn	0.55	0.02	Negative
		α-tocopherol	0.54	0.02	Negative
		CP	0.54	0.02	Negative
		Mg	0.53	0.03	Negative
		ME	0.52	0.03	Negative
		K	0.51	0.03	Negative
		Ca	0.50	0.03	Negative
		Zn:Cu	0.48	0.04	Positive
		Fe	0.47	0.04	Negative
		P	0.37	0.08	Negative
		Fat	0.36	0.09	Negative
		Ca:P	0.34	0.10	Negative
GSH-Px	Blood	P	0.56	0.01	Positive
		Mg	0.43	0.04	Positive
		β-carotene	0.39	0.05	Positive
		Ca	0.37	0.06	Positive
		α-carotene	0.36	0.07	Positive
FRAP	Blood	---			
	Seminal Fluid	α-tocopherol	0.73	0.003	Negative
		TDF	0.62	0.01	Negative
		Fe	0.60	0.01	Negative
		Ca	0.60	0.01	Negative
		DM	0.55	0.02	Negative
		Mn	0.54	0.02	Negative
		P	0.54	0.02	Negative
		OM	0.53	0.03	Negative
		S	0.52	0.03	Negative
		ME	0.48	0.04	Negative
		Zn	0.44	0.05	Negative
		CP	0.41	0.06	Negative
		Cu	0.41	0.06	Negative
		Fat	0.38	0.07	Negative
		K	0.39	0.07	Negative
		Zn:Cu	0.34	0.10	Positive

Table 3. Regression analyses of blood, seminal fluid, and weekly dietary intakes with total sperm count and percent normal sperm morphology.

Measure	Sample Type	Variable	R^2	P-Value	Association
Total Sperm Count	Diet	Lauric Acid	0.72	0.0001	Positive
	Blood	GPx	0.61	0.007	Positive
	Diet	ω-3	0.53	0.003	Positive
	Diet	Linolenic acid	0.52	0.003	Positive
	Diet	ω-3:ω-6	0.47	0.007	Positive
	Diet	Docosenoic Acid (22:1)	0.41	0.01	Positive
	Serum	Copper	0.36	0.03	Negative
	Diet	Phosphorus	0.33	0.03	Positive
	Diet	Lignoceric acid	0.33	0.04	Negative
	Diet	Retinol ester	0.32	0.04	Positive
	Serum	Phosphorus	0.30	0.05	Negative
	Diet	Zn:Cu	0.27	0.06	Positive
	Diet	DPA 22:5 n3	0.26	0.07	Positive
	Diet	Retinol	0.24	0.07	Positive
	Diet	Crude protein	0.24	0.07	Positive
Percent Normal Morphology	Seminal Fluid	SOD	0.90	0.001	Negative
	Seminal Fluid	PC	0.76	0.02	Negative
	Diet	Copper	0.63	0.004	Positive
	Diet	Iron	0.42	0.02	Positive
	Blood	β-cryptoxanthin	0.41	0.03	Negative
	Diet	Zn:Cu	0.38	0.03	Negative
	Blood	Retinol ester	0.37	0.04	Negative
	Blood	α-tocopherol	0.37	0.04	Negative
	Diet	Manganese	0.36	0.04	Positive
	Diet	α-tocopherol	0.35	0.04	Positive
	Diet	Sulfur	0.34	0.05	Positive
	Blood	β-carotene	0.33	0.05	Negative
	Diet	Arachidic acid	0.29	0.07	Positive
	Diet	Ca:P	0.26	0.09	Positive
	Blood	TBARS	0.25	0.10	Positive
	Blood	PC	0.24	0.10	Positive