

NC STATE UNIVERSITY

COLLEGE OF VETERINARY MEDICINE



Second Annual

Crissey Zoological Nutrition Symposium

Raleigh, North Carolina

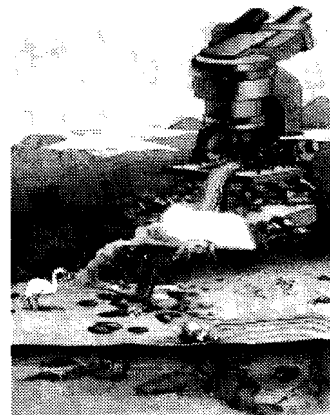
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Raleigh, North Carolina
December 10 and 11, 2004

Susan D. Crissey, Ph.D.
December 12, 1951 - November 23, 2002

Sue Crissey earned her B.S. and M.S. degrees in human nutrition from Michigan State University and spent four years with the FDA before accepting a scholarship from the University of Maryland to pursue a Ph.D. in animal nutrition. She completed a post-doctoral fellowship at the Smithsonian Institution's Conservation Research Center in Front Royal, Virginia, and began field work studying howler monkeys in Venezuela. From there, she joined the staff of the Brookfield Zoo in Chicago where she developed and led their nutrition programs.

Sue continued as Director of Nutrition for Brookfield Zoo until her death. It was much to North Carolina State University's advantage when Sue moved to Burgaw, North Carolina, to be with her husband, Chris Smith. She accepted an appointment as adjunct assistant professor in the Department of Clinical Sciences and taught many students the basics of zoological nutrition. Sue was an energetic and engaging lecturer who could draw on her work with nutritional diseases in species that included rhinoceros, wild felids, howler monkeys, golden marmosets, bottlenose dolphins, Micronesian kingfishers, and many more, to illustrate her talks and discussions. Sue published over 100 scientific papers including several seminal topical reviews. In 2002, she was awarded the Duane E. Ullrey Achievement Award by the American Association of Zoo Veterinarians for her distinguished work.

Sue loved her North Carolina farm, and maintained a significant menagerie of zoo retirees and castaways there, commuting from her home in Burgaw to Chicago to manage her zoo duties, and traveling to Raleigh at the drop of a hat to teach. Sue was a meticulous scientist whose enthusiastic joys of teaching and insistence on "good science" have become part of those who were lucky enough to be around her for any length of time. Future generations of zoological nutritionists are richer for having been, but poorer for not knowing her.

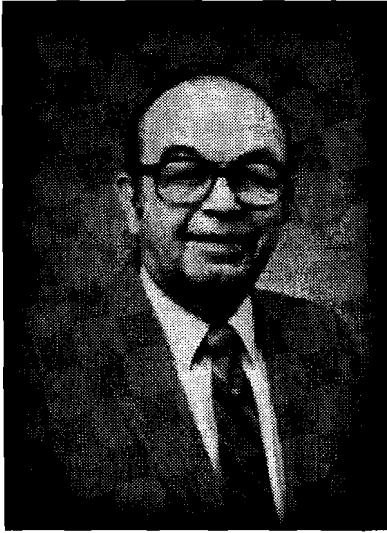
"I don't know that I was a great teacher, but in almost everything I did, I tried to encourage others to look for opportunities to be helpful to people and to appreciate our natural world."

- Sue Crissey, 2002



Susan D. Crissey

The Key Note Speaker



Dr. C. Edward Stevens (B.S. , D.V.M.,M.Sc., Ph.D. University of Minnesota) is a Professor Emeritus and the former Associate Dean and Director for Research and Graduate Studies at the College of Veterinary Medicine, North Carolina State University. A veterinarian and a research physiologist, Dr. Stevens has studied the comparative physiology of the digestive tract of vertebrates throughout a long and illustrious career. Much of his work has centered on the rumenal physiology and its relation to digestion and nutrition. He has served as the President of the American Association of Veterinary Physiologists and pharmacologists, been a member of the National Agricultural Research Council, and was the founding research dean for the NCSU College of Veterinary Medicine. His book, *Comparative Physiology of the Vertebrate Digestive System*, now in a second edition and a CD-ROM version, is a landmark work and a staple of comparative digestive physiology courses around the world.

Representative Publications of Interest

Stevens, C.E., and B. K. Stettler, Factors affecting the transport of volatile fatty acids across rumen epithelium. *Am. J. Physiol.* 210:365-372, 1966.

Stevens, C.E. *Comparative Physiology of the Digestive System*. In: *Dukes' Physiology of Domestic Animals*. 9th Ed. Ed. M.J. Swenson, pp 216-232, Comstock, Cornell University Press: Ithaca, 1977.

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**Second Annual Crissey Zoological Nutrition Symposium
Schedule of Events and Table of Contents**

Friday, December 10, 2004

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2:00 – 2:15pm 3	Michele Gaffney, MS	Intake and Apparent Digestibility of an Extruded Particle Diet for Felids by Pallas' Cats (<i>Otocolobus manul manul</i>)	17
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4:15 – 5:00pm	Conference Closing Remarks		

CONSIDERATIONS FOR MEAT DIETS FED TO ZOO ANIMALS

Barbara A. Lintzenich, M.S.^{1*}, Kerri A. Slifka, M.S.¹, Ann M. Ward, M.S.²

¹Daniel F. and Ada L. Rice Conservation Biology and Research Center, Chicago Zoological Society, Brookfield Zoo, Brookfield, IL 60513, balintze@brookfieldzoo.org
keslifka@brookfieldzoo.org

²Fort Worth Zoo, 1989 Colonial Parkway, Fort Worth TX 76110, award@fortworthzoo.org

Many factors are considered in selecting appropriate raw meat diets for zoo carnivores and monitoring their quality. Currently, both horse and beef based raw meat diets are available. These diets have been designed especially for meat-eating animals. Factors that need to be considered are: 1) nutrient composition of the diets, 2) procedures/protocols in the processing of the diets, 3) quality control analysis of the diets, and 4) consumption by the animals.

The meat diets available are all tailored to meet general nutrient requirements of carnivorous animals (see Table 1).² Nutrient composition of the meat diets are typically compared to probable nutrient requirements developed for that particular species (see Table 2).^{1,5} The companies usually provide a maximum and/or minimum in a guaranteed analysis of a few nutrients. A more thorough nutrient analysis is recommended for review to have the most complete comparison. After the product is chosen, discussions with or even site visits to the manufacturing company are encouraged. General knowledge of how the product is developed, processed and an overview of quality control analysis is important information to obtain from the manufacturer. A list of 16 questions was developed at BZ and FWZ to become more familiar with the manufacturing of the products.^{4,6} The questions are: 1) Do you use HACCP?, 2) What kind of training do you provide for the employees?, 3) Are the staff certified for sanitation/food handling? If so by what agency?, 4) Is there a regulatory body that inspects your operation? If so how often?, 5) What is the inspection procedure (evaluation criteria) for the raw ingredients entering the plant?, 6) What is the inspection procedure (evaluation criteria) for the finished product?, 7) How do you trace ingredients (link) to the finished product?, 8) Please describe your product recover system?, 9) How do you handle customer complaints?, 10) What is schedule for preventative maintenance?, 11) What analysis do you conduct in house (lab)?, 12) For your lab, what quality control practices do you have?, 13) What analyses do you conduct in labs outside of the plant and how often?, 14) What is your freezing method?, 15) What temperature is the product kept during process?, 16) Do you utilize lot number, batch numbers, etc. and is this on the label?

Once the product is purchased it is still important to monitor nutrient composition of the diet (see Table 3). It is important to monitor protein, fat, energy, minerals, and fat-soluble vitamins. A representative sample of the meat diet should be obtained and either analyzed yearly.³ Additionally, raw meat diets should be analyzed for microbial content. Allen et al (1999) list microbiological guidelines for raw meat-based diets. Finally, the animal should consume the diet and maintain appropriate body condition on the diet. Regular monitoring of consumption is essential.

References:

- ¹Association of American Feed Control Officials (AAFCO). 2004. Dog and Cat Nutrient Profiles. Published by The Association of American Feed Control Officials. Oxford, IN. Pp:128-143.
- ²Allen, M.E., D.E. Ullrey, and M.S. Edwards. 1999. The Development of Raw Meat-based Carnivore Diets. Proc. Am. Assoc. Zoo Vets, Columbus OH. Pp:317-319.
- ³Bernard, J.B., J.L. Dempsey, D.E. Ullrey, A.M. Ward, and M.E. Allen. 1999. Quality Control of Feedstuffs: Nutrient Analysis. Technical Paper 010. www.NAGonline.net
- ⁴Crissey, S.D., K.A. Slifka, P. Shumway, and S.B. Spencer. 2001. Handling Frozen/Thawed Meat and Prey Items Fed to Captive Exotic Animals: A Manual of Standard Operating Procedures. U.S. Department of Agriculture, Agricultural Research Service, National Agriculture Library. Pp:1-23.
- ⁵National Research Council. 2004. Nutrient Requirements of Cats and Dogs. National Academy Press, Washington, D.C.
- ⁶Silliker Laboratories Group, Inc., Silliker Standards for Food Safety: Good Practices Requirements for Food Processing Facilities. Homewood, IL. Pp:1-14.

Table 1. Product Nutrient Concentrations on a dry matter basis except moisture

Nutrient	Value	Nutrient	Value
Moisture, % max	70	Crude Protein, % min	30
Crude Fat, % min	10	Crude Fiber, % max	3
Lysine, %	4.3	Taurine, %	0.3
Calcium, %	1.3	Phosphorus, %	1.2
Magnesium, %	0.09	Zinc, mg/kg	110
Vitamin A, IU/kg	14,000	Vitamin E, mg/kg	427

Table 2. Probable Nutrient Requirements for Cats on a dry matter basis

Nutrient	NRC	AAFCO	Nutrient	NRC	AAFCO
Protein, %	16-30	26-30	Calcium, %	0.52-0.8	0.6-1
Fat, %	9-15	9	Phosphorus, %	0.48-0.72	0.5-0.8
Vitamin A, IU/g	2.8-7.5	5-9	Magnesium, %	0.016-0.04	0.04-0.08
Vitamin D3, IU/g	0.12-0.25	0.5-0.75	Potassium, %	0.27-0.52	0.6
Vitamin E, mg/kg	30-38	27	Sodium, %	0.065-0.14	0.2
Thiamin, mg/kg	4.4-5.5	5	Iron, mg/kg	70-80	80
Riboflavin, mg/kg	3.4-4.25	4	Zinc, mg/kg	50-75	75
Niacin, mg/kg	34-42.5	60	Copper, mg/kg	4.5-8.4	5-15
Pyridoxine, mg/kg	2-2.5	4	Manganese, mg/kg	4.8	7.5
Folacin, mg/kg	0.6-0.75	0.8	Iodine, mg/kg	2.2	0.35
Choline, mg/kg	2040-2550	2400	Selenium, mg/kg	0.12-0.4	0.1

Table 3. Nutrient analysis of raw meat diets compared to guaranteed analysis on a dry matter basis.

Nutrient	Product 1 ^a		Product 2 ^b		Product 3 ^c		Product 4 ^d		Product 5 ^e	
	Guar ^f	Test ^g	Guar ^f	Test ^g	Guar ^f	Test ^g	Guar ^f	Test ^g	Guar ^f	Test ^g
Dry Matter, %	No Spec	29.6-32.4	No Spec	30.2-40.6	No Spec	32.4-35.3	No Spec	63.8	30	34.8-35.2
Crude Protein, %	30 Min	64.1-69.7	30 Min	23.9-48.8	50 Min	56.1-63.6	49.3 Min	53.9	5.4	52.3-53.1
Crude Fat, %	10 Min	11.4-14.4	40 Max	36.6-38.4	30 Min	21.0-29.5	31.6 Min	30.3	3.0	34.2-33.5
Ash, %	8 Max	8.2	8 Max	6.3	No Spec	5.1-6.2	11.8 Max	ND	0.97	7.1-7.5
Calcium, %	0.8-1.6	1.5-1.5	0.8-1.6	1.1-1.3	0.8 Min	1.3	1.6 Min	1.9	0.18 min	1.8
Phosphorus, %	0.6-1.2	1.1-1.3	0.6-1.2	0.9-1.0	0.7 Min	0.8	1.3 Min	1.1	0.11 min	1.1
Magnesium, %	0.5-1.0	0.1-0.1	0.5-1.0	0.1-0.1	0.1 Min	0.1	No Spec	0.1	No Spec	0.8
Potassium, %	0.5 Min	1.2-1.2	0.5 Min	0.8-0.9	No Spec	1.0	No Spec	0.9	No Spec	0.8
Sodium, %	0.2 Min	0.6	0.2 Min	0.5-0.5	No Spec	0.2	No Spec	0.3	No Spec	0.5
Iron, ppm	80 Min	130-142	80 Min	86.2-96.9	160	124	No Spec	418	No Spec	151
Zinc, ppm	75 Min	112-139	75 Min	82.1-97.2	120	180	No Spec	101	No Spec	199
Copper, ppm	5 Min	21.7-22.8	5 Min	12.5-18.2	No Spec	ND	No Spec	ND	No Spec	50.0
Manganese, ppm	7.5 Min	10.0-10.6	7.5 Min	7.1-8.6	No Spec	27.7	No Spec	29.0	No Spec	23.0
Vitamin A, IU/g	10 Min	12.3	10 Min	ND	11	9.1-52.0	10.5 Min	ND	No Spec	9.5
Vitamin E, IU/kg	200 Min	473	200 Min	ND	200	143-289	No Spec	ND	No Spec	251
Vitamin D, IU/g	1 Min	ND	1 Min	ND	2.2	1.5-2.8	1 Min	ND	No Spec	1.5
Taurine, ppm	No Spec	ND	No Spec	ND	2000	1970-2010	No Spec	ND	No Spec	3090

Products: ^aDallas Crown 95/5, ^bDallas Crown 85/15, 2000 W. Fair, Kaufman, Texas 751421 ^cMilliken Feline, 3447 Kennedy Road, Unit 1, Scarborough, Ontario Canada M1V 3F1; ^dNebraska Feline, P.O. Box 550, North Platte, Nebraska 69103-0550; ^eNatural Balance Zoo Carnivore, 12924 Pierce Street, Pacoima, California 91331.

^fGuaranteed analysis provided by manufacturer.

^gResults of tests run as part of a quality control program by Brookfield and Fort Worth Zoos. Same numbers in a range indicate same value from repeated sampling. One number indicates analysis performed on one sample. Some nutrients were not guaranteed by the manufacturer (No spec) or not measured by the zoos (ND).

INTAKE AND APPARENT DIGESTIBILITY OF A BEEF-BASED CARNIVORE DIET BY A SUMATRAN TIGER (*PANTHERA TIGRIS SUMATRAE*)

Michele Gaffney, MS*, Michael L. Schlegel, MS, PhD[†], and Mark S. Edwards, PhD

Zoological Society of San Diego, POB 120551, San Diego, CA 92112-0551 USA
medwards@sandiegozoo.org
schlegel@animal.ufl.edu
migaffney@aol.com

[†]Current affiliation: Department of Animal Sciences, University of Florida, POB 110910, Gainesville, FL 32611 USA

Significant changes in the types of raw meat diets available for use in feeding captive carnivores have occurred over the past five years. These changes have resulted in multiple options regarding source ingredients, formulations, and packaging.¹ However, due to their relatively recent availability, intake and utilization of many of these diets have not been objectively evaluated. As part of the preventive health program, routine food intake, fecal consistency, and food utilization was evaluated in this species to objectively assess its use in the applied nutrition program.

Methods

Food intake and total fecal output was quantified for male Sumatran tiger (*Panthera tigris sumatrae*) for three consecutive days. The 12-year-old animal was housed in quarantine facilities throughout the collection period. The animal's body weight (BW) was 100.5 and 101.5 kg at the beginning and completion of the collection period, respectively.

A fresh, beef-based diet for carnivores (Table 1; Natural Balance[®] Zoo Carnivore Diet 5; Dick Van Patten's Natural Balance Pet Foods, Inc. Zoological Division, Pacoima, CA 91331) was the sole diet offered *ad libitum* for 21 days prior to, and during the three-day collection period. Food items offered and refusals collected following 24 h were weighed. Water was available *ad libitum*. Physical characteristics and consistency of all fecal samples were scored using a 0-100 scale, where moisture content of the excreta decreases as the score increases from 0 to 100.

Food and fecals were dried to a constant weight in a forced-air drying oven (50°C).⁴ Dry matter (DM) content of those samples was determined by drying a weighed sample in a vacuum (27 to 30 mm Hg) drying oven at 100°C.² Gross energy (GE) content of food and fecals was determined by isoperibol calorimetry.⁵ Apparent digestibility of dry matter and energy were calculated according to Van Soest.⁶

Results And Discussion

Intake of the fresh meat diet was $845 \text{ g DM}\cdot\text{d}^{-1}$ (0.82% BW). Apparent dry matter digestibility (ADMD) of the diet was $82.2 \pm 0.51\%$.

Gross energy content of the diet consumed was $5.91 \text{ kcal}\cdot\text{g DM}^{-1}$. The apparent GE digestibility of the diet by this specimen was $87.1 \pm 0.43\%$. During the three-day collection period, energy intake was $4991 \text{ kcal GE}\cdot\text{d}^{-1}$ ($156.7 \text{ kcal GE}\cdot\text{BW}_{\text{kg}}^{-0.75}$) or $4348 \text{ kcal DE}\cdot\text{d}^{-1}$ ($136.5 \text{ kcal DE}\cdot\text{BW}_{\text{kg}}^{-0.75}$).

Physical characteristics of fecals produced during the collection period were in a desirable range (score = 75) for the species. Fecal moisture content was 77.2%.

Understandably, these results are of limited significance due to inadequate number of specimens ($n = 1$), and relatively short collection period. However, these findings are similar to those of a larger study examining felid utilization of fresh-meat carnivore diets with variable fiber sources: wood cellulose, beet pulp, and a fiber blend of wood cellulose and fructooligosaccharides (FOS)³. The fiber content of those test diets was 12.3-17.1% total dietary fiber (TDF).

The diet used in this study, which includes beet pulp as the primary fiber source, contained 3% neutral-detergent fiber (NDF) and 2% acid-detergent fiber (ADF), significantly lower fiber concentrations than the diet in the comparative study. Total dietary fiber concentrations have not been determined for the diet used in this study.

Across all three diets used in the comparative study, large cats (African lions, *Panthera leo krugeri*; and Sumatran Tigers) consumed 864 to 881 $\text{g DM}\cdot\text{d}^{-1}$ (0.77-0.78% BW), which is slightly lower than intake levels in this report.³ Conversely, ADMD of the three higher fiber test diets range from 62.8-65.8%, which is lower than 82% ADMD reported here.³ Additionally, as ADMD decreased with increased fiber levels, DE content was lower and ranged from 2.7 – 4.9 $\text{kcal}\cdot\text{g DM}^{-1}$ which is lower than the $5.15 \text{ kcal}\cdot\text{g DM}^{-1}$ determined in the current study.

Although lower ADMD would be anticipated when carnivores are fed a higher fiber diet when contrasted to a lower fiber diet, a subsequent increase in intake is typically observed to compensate for the overall reduction in net utilization. These differences were not seen during the collection period of this assessment, but may have been appreciated if the evaluation had been continued over a comparable length of time.

In conclusion; the fresh, beef-based diet for carnivores was sufficient to support the weight of the animal during the collection period, and throughout the quarantine phase. Additional species-specific response criteria need to be objectively evaluated for new formulations of meat-based carnivore diets to refine management techniques and improve dietary husbandry protocols.

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Table 1. Selected calculated nutrient content of a fresh, beef-based diet for carnivores^a.

% DM					
Moisture	Crude Protein, %	Ether Extract, %	Ca, %	P, %	Taurine, %
31.0	61.3	22.3	1.9	1.3	0.65

^aNatural Balance[®] Zoo Carnivore Diet 5; Dick Van Patten's Natural Balance Pet Foods, Inc. Zoological Division, Pacoima, CA 91331; www.naturalbalanceinc.com

INTAKE AND APPARENT DIGESTIBILITY OF AN EXTRUDED PARTICLE DIET FOR FELIDS BY PALLAS' CATS (*OTOCOLOBUS MANUL MANUL*)

Mark S. Edwards, PhD, Karen J. Lisi, MEM, MS, and Michele Gaffney, MS*

Zoological Society of San Diego, POB 120551, San Diego, CA 92112-0551 USA
medwards@sandiegozoo.org
klisi@sandiegozoo.org
migaffney@aol.com

Many adult Pallas' cats (>80%) in North American zoos are chronically seropositive for *Toxoplasmosis gondii* antibodies.³ During pregnancy, maternal immune responses apparently are not protective in Pallas' cats, unlike other cat species with prior *T. gondii* exposure.³ Neonatal Pallas' cats could be exposed to *T. gondii* cysts from multiple sources, including diet items such as whole animal prey and fresh meat diets.⁷

In an effort to reduce neonatal mortality associated with *T. gondii* exposure, multiple litters of Pallas' cats born at the San Diego Zoo have been transferred for hand-rearing in a controlled nursery environment. Initially, as kittens begin to consume solids, they are weaned from formula to a combination of canned kitten and extruded feline diets. Ultimately, the juveniles are weaned to a sole diet of the extruded feline diet.

As part of a preventive health program, routine food intake, fecal consistency and food utilization were evaluated in this species to objectively assess this management strategy in the applied nutrition program.

Methods

Food intake and total fecal output were quantified for a pair of male Pallas' cats (*Otocolobus manul manul*) for five consecutive days. The two animals were 16-month-old littermates, housed in the same enclosure 7 months prior to, and throughout the collection period. Mean body weight at the completion of the collection period was 4.1 ± 0.6 kg.

An extruded diet for felids (Table 1; Mazuri[®] Exotic Feline, Small (5M54)) was the sole diet offered *ad libitum* for 7 months prior to, and during the collection period. Food items offered and refusals collected following 24 h were weighed. Water was available *ad libitum*. Fecal material, representing combined output of the animals over a 24h period, was collected daily. Physical characteristics and consistency of all fecal samples were scored using a 0-100 scale, where moisture content of the excreta decreases as the score increases from 0 to 100.

Food and fecals were dried to a constant weight in a forced-air drying oven (50°C).⁴ Dry matter content of those samples was determined by drying a weighed sample in a vacuum (27 to 30 mm Hg) drying oven at 100°C.² Gross energy (GE) content of food and fecals was determined by isoperibol calorimetry.⁶

Average food intake and fecal output were determined by the total quantity of each measurement divided by the number of animals in the enclosure. Apparent digestibility of dry matter and energy were calculated according to Van Soest.⁸

Results And Discussion

Dry matter intake (DMI) of the extruded particle diet was $98.3 \text{ g}\cdot\text{d}^{-1}$ (2.40% BW). Apparent dry matter digestibility (DMD) of the diet was $77.4 \pm 5.76\%$.

Gross energy content of the diet was $5.55 \text{ kcal}\cdot\text{g}^{-1}$. Apparent GE digestibility was $82.2 \pm 4.59\%$. Thus, the digestible energy (DE) content of this diet for the specimens tested was $4.56 \text{ kcal DE}\cdot\text{g}^{-1}$. During the collection period, energy intake was $545 \text{ kcal GE}\cdot\text{d}^{-1}$ ($189 \text{ kcal GE}\cdot\text{BW}^{-0.75}$) or $448 \text{ kcal DE}\cdot\text{d}^{-1}$ ($156 \text{ kcal DE}\cdot\text{BW}^{-0.75}$).

Intake of extruded feline diet was adequate to support body weight of the two juvenile males throughout the collection period. Absolute DMI (98.3 vs. $104.7 \text{ g}\cdot\text{d}^{-1}$) and DMI, %BW (2.4 vs 3.4% BW) were both lower than consumption reported in sand cats (*Felis margarita*) fed the same diet.⁴

Apparent DMD of the diet in this evaluation was slightly greater than that observed in sand cats fed the same diet (77.4 vs. 72.7%).⁴ Apparent GE digestibility was similarly higher (82.2 vs. 76.8%).³ The lower energy digestibility observed in sand cats equates to a DE intake of $446 \text{ kcal DE}\cdot\text{d}^{-1}$ ($191 \text{ kcal DE}\cdot\text{BW}^{-0.75}$) that is comparable to the $448 \text{ kcal DE}\cdot\text{d}^{-1}$ ($156 \text{ kcal DE}\cdot\text{BW}^{-0.75}$) observed in this assessment.

Physical characteristics of fecal material were in a desirable range (score = 75) for the species. This is of particular interest, since others have reported extruded diets, when consumed as the sole component of the diet, having negative effects on fecal consistency.

Application of this diet and feeding strategy has been effective in creating an environment that minimizes exposure of neonates to *T. gondii* cysts and supports normal development to adulthood.

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Table 1. Selected calculated nutrient content of an extruded diet for felids^a.

Moisture, %	DM				
	Crude Protein, %	Ether Extract, %	Ca, %	P, %	Taurine, %
7.5	38.9	21.6	1.7	1.4	0.28

^aMazuri[®] Exotic Feline, Small 5M54, www.mazuri.com

SWITCHING FROM RAW MEAT TO A PROCESSED DIET IN PALLAS' CATS

Suzanne Kennedy-Stoskopf, DVM, PhD, Diplomate American College of Zoological Medicine

North Carolina State University, College of Veterinary Medicine,
4700 Hillsborough Street, Raleigh, NC 27606
suzanne_stoskopf@ncsu.edu

Pallas' cats (*Otocolobus manul*), both neonates and adults, have experienced significant mortalities from toxoplasmosis in captivity. Pathological findings demonstrate that long-term, seropositive adults experience reactivation of encysted bradyzoites and subsequent tissue necrosis from rapidly replicating tachyzoites. Epidemiology of neonatal deaths suggests *in utero* transmission or possibly lactogenic transmission. Although there has been speculation that Pallas' cats are immunocompromised, there is no conclusive evidence for this. Felids are most readily infected with *Toxoplasma gondii* by consuming prey or raw meat containing encysted bradyzoites. Traditionally, captive, wild felids are fed a raw meat diet and often have the opportunity to capture potentially infected prey species within their enclosures. Switching to a processed diet would eliminate a possible source of infection for the Pallas' cat.

In 2000, 4.2 Pallas' cats, IgG positive for *T. gondii*, were donated to North Carolina State University College of Veterinary Medicine (NCSU CVM) to establish a research colony. One goal of the colony was to produce specific pathogen free (SPF) Pallas' cat kittens for the SSP. In 2002, six kittens were born to a female that arrived in 2001 with an IgG titer of 1:64 to *T. gondii*. The kittens were pulled at birth and cross-fostered onto nursing, SPF domestic cat queens. They were weaned onto Iams Kitten and remained on Iams as adults. The Iams feline diet appeared to support growth and eventual reproduction in these cats.

To determine if a commercial source of cat food would foster optimal health and reproduction in Pallas' cats, a collaborative study funded by Iams was begun November 2003, between North Carolina State University (S. Kennedy-Stoskopf), Center for Research of Endangered Wildlife (W. Swanson), and the St. Louis Zoological Park (E. Dierenfeld). The study groups were 7 Pallas' cats that had been fed Iams since birth and 7 animals that had always been on a raw horsemeat based diet. This is an ongoing study scheduled for completion in March 2005. Study parameters include blood collection every 4 months for CBC, serum chemistries, vitamins A, D, and E, taurine, elemental concentrations (Ca, P, Fe, Na, K, Se, Co, Cu, Mg, Zn), and serum fatty acid components. Urine samples collected at the same intervals will be analyzed for pH, minerals, and creatinine. Digestibility studies using chromic oxide will be evaluated for both diets. To evaluate reproductive health, semen will be collected 1-2 times during the breeding season (Feb – April), and evaluated for sperm concentration, % motility, % abnormal sperm forms and total volume, and spermatozoa will be analyzed for fatty acid content. Dental scores were also made on all animals in the study. Ten months into the study, the Pallas' cats on horsemeat were switched to Iams. This abstract describes how this was accomplished.

The switch began using canned Iams feline beef only. The absolute weight of food fed each cat did not change. The canned Iams was added initially at 10% of the diet (ie. a cat fed 5 oz of diet would initially get 4.5 oz horsemeat and 0.5 oz Iams). Cats were fed each proportion of

diets for 3 days before increasing the amount of canned Iams by another 10%. When the cats reached 70% Iams and 30% horsemeat, then the dry food, Iams Chicken and Rice Formula, was introduced beginning with 1 tablespoon mixed well into the diet. An additional tablespoon was added every 3 days until 4 T. or ¼ cup was reached. The goal was to achieve 20-25% of the 5-7 oz of canned diet as dry food. These cats had traditionally been fed SPF mice 4 times a week. The decision was made to continue this practice.

Five adult cats (3.2) participated in the dietary switch. Two founder cats (1.1, > 10 yrs of age) died suddenly from meningoencephalitis due to *T. gondii* early in the study while still on horsemeat. All cats except one readily accepted the introduction of a 10% addition of canned Iams feline beef into their diet every 3 days. The fifth cat required 6-7 days. Only one cat readily accepted the addition of 1 T. dry food into the diet every 3 days. Three cats were given 7 days before dry food was increased. The fifth cat that had readily accepted the increase to 70% Iams and 30% horsemeat stopped eating well with the introduction of dry food. Dry food was discontinued and the diet dropped back to 60% Iams and 40% horsemeat. While the other cats had completely converted to a mixture of canned and dry diet within 2 months, this cat took 3 1/2 months.

Although the dietary switch occurred better than expected, several health issues developed that were cause for concern. Three weeks into the switch, one cat developed upper respiratory signs. All cats in the colony have a history of clinical feline herpesvirus-1 (FHV-1) or positive PCR result for FHV-1 from ocular conjunctiva. By 8 weeks, all 5 cats were experiencing upper respiratory signs. While it is not unusual for one or two cats to be symptomatic periodically, they have never been symptomatic simultaneously. While it was assumed that FHV-1 was responsible, attempts at virus isolation were negative. Three cats required antibiotic treatment before resolution of clinical signs. This is also highly unusual as antibiotics seldom need to be administered to Pallas' cats with prolonged upper respiratory signs in the NCSU colony. In addition, the Pallas' cat that accepted the dietary change most readily experienced intermittent vomiting and soft stools weeks 5-9 of the diet switch. These resolved without treatment. In general, clinical signs did not adversely impact appetite.

More disconcerting was the weight loss experienced between 7/20/04 when dental scoring was performed and 10/01/04 when the cats were anesthetized for FHV-1 isolation. The average weight loss was 0.60 kg \pm 0.32 kg, range 0.24-1.12 kg. The 2 females lost less than the males, and the extreme weight loss was the one male that took over 3 months to switch his diet. Normally in the fall, these cats are putting on weight in preparation of the breeding season. Mice were increased from 4 times a week to daily. However, a review of the Pallas' cats' historical weights during this time of year suggest that they are in line to weights recorded when they were fed a horsemeat based product from a different supplier. Currently, all cats are eating well and appear healthy. Expect a complete report of the results of this study at next year's Crissey Zoological Nutrition Symposium.

WHAT IS A CONUNDRUM?

Co nun drum n. 16th century Oxford University L slang for pendant, whim, et.; early sp. quonundrum 1. a riddle whose answer contains a pun 2. any puzzling question or problem **SYN** mystery (Webster's New World College Dictionary).

For the purposes of the Crissey Zoological Nutrition Symposium, we are using the word in the sense of the second definition, a puzzling question or problem. The purpose of the symposium is to bring together inquiring minds interested in various aspects of comparative nutrition to foster collaborative and progressive interaction. We, the organizers, decided the symposium also offered an excellent opportunity to glean insight from many view points about questions in zoological nutrition that don't have easy answers. Our hope is that everyone at the symposium will join in on these green light thinking exercises.

At the end of each session, a conundrum will be posed. These are also in the proceedings book, for those that like to work ahead. Our hope is the question briefly posed at the end of each session will spark discussion and conversation during the ensuing break or through out the evening. At the next session, the first 15 minutes of the session will be devoted to discussion and comments about the conundrum presented in the last.

Our goal is not so much to reach any definitive answers to the conundra presented, but rather to identify what needs to be known and what steps need to be taken to begin to find satisfactory answers to the questions posed. In a very real way, we will be generating a road map of research ideas of a sort. We will attempt to make a coherent brief synopsis of the discussions of each conundrum, which we hope to distribute to the participants of this year's symposium by email, hopefully not too long after the symposium closes. We invite refinement of the summaries and additional ideas to add to what will be published in the following year's proceedings book as a record of our thoughts.

In addition, we would like to solicit conundra from the symposium participants for discussion next year. Interesting questions, contradicting dogmas, are all welcome as possible conundra for discussion next year. Submit your ideas by email to Kimberly Ange (Kimberly_Ange@ncsu.edu) or Michael Stoskopf (Michael_Stoskopf@ncsu.edu) as they come to mind over the next year.

CONUNDRUM I

Many consider it clinically important to reduce protein intake for animals in an effort to spare the kidney when they are fighting an infectious disease that targets the kidney. However, it is also considered clinically important to healing to have a positive nitrogen balance. Reducing protein intake for obligate carnivore usually means reducing or stopping feed entirely. When faced with a diagnosis of an infectious disease that impacts the kidneys in an obligate carnivore that is still feeding on their own (example: leptospirosis in a pinniped), is better to withhold feed, or to continue feeding the animal through the course of treatment?

THE PRACTICAL CHALLENGES OF IMPLEMENTING A ZOO NUTRITION PROGRAM

M. E. Hile, MPS, MSc

North Carolina Zoo
liz.hile@ncmail.net

The challenge of a zoo nutrition program is to provide optimal nutrition to the animals in its care, while attempting to address the psychological needs associated with the behavior of feeding. The “production” goals of a zoo nutrition program differ vastly from those of the pet or livestock industry. “Optimal” must encompass coordinating feeding programs with the captive animal management goals of reproduction, longevity, and behavioral normality by attempting to meet psychological and physiological needs of species that have evolved under diverse environmental circumstances, to occupy specialized niches, with the ability to make diet choices according to seasonal, physiologic, environmental, or individual needs.

Although diets can be formulated that are similar in nutritional content to an animal’s natural diet through substitution, not all captive animals are capable of accepting substitutions. Taste, texture, odor, size, shape, color, and movement are important factors and can be more compelling than the actual nutrient content of the diet. The scope of a zoo nutrition program encompasses the challenges of ration formulation, procurement, storage, and handling of very unique feed stuffs for a very unique clientele. It also must deliver optimal nutrient content to collection animals and attempt to incorporate animal’s dietary preferences and desired presentation, as well as support animal management, training, and enrichment programs to sustain psychological welfare. A zoo’s “production” goals of reproduction, longevity, and behavioral normality cannot be met solely by presenting captive wildlife with adequately balanced rations, which is a challenge in itself due to the limited information available. Considering that wild animals, humans included, spend a majority of their waking hours in the pursuit food, the nutrition program can be considered the cornerstone of managing wildlife in captivity. The true challenge is replicating enough of the natural environment on a small scale to satisfy the animal’s needs for physiological and psychological welfare. No single discipline addresses all these needs. The truly challenging and exciting aspect of the captive management field is that we must continue to research new information and partner with the many disciplines that offer insight into improving the captive management of wildlife.

RESEARCH UPDATE ON MOLECULAR DETECTION OF PREY IN CARNIVORE FECES

AE Acton*, DVM^{1,2}, CA Whittier, DVM^{1,2}, MK Stoskopf, DVM, PhD, DACZM^{1,2}

¹Environmental Medicine Consortium, ²Department of Clinical Sciences, College of Veterinary Medicine, 4700 Hillsborough St., North Carolina State University, Raleigh, NC 27606, USA,
aeacton@ncsu.edu

Last year we reported on the successful use of species-specific mtDNA probes to detect white-tailed deer in red wolf feces up to 3 days following a single feeding event. PCR assays could identify prey in the feces in the absence of gross evidence. Subsequent trials have demonstrated intermittent prey recovery as long as 6 days after a single feeding event. Here we report the results of an ongoing study of various sample storage techniques that could influence assay interpretation. The challenges encountered when developing separate prey detection assays for 2 other common dietary items will also be discussed.

CYSTINE UROLITHIASIS POSSIBLY ASSOCIATED WITH A FELINE NOVEL PROTEIN DIET IN DOMESTIC FERRETS: TWO CASES

Dan H. Johnson, DVM, Christine Eckermann-Ross, DVM.

Avian and Exotic Animal Care, PA, Raleigh, NC. Adjunct Assistant Professors, Dept. of Clinical Sciences, NC State College of Veterinary Medicine.

Urolithiasis was diagnosed in two Ferrets that were being fed a novel protein diet intended for use on cats.* In both cases, urinary stone analysis was performed and the urinary stones were found to be composed of cystine. These cases, along with observations made by others, suggest that novel protein diets intended for use on cats may contribute to the formation of cystine uroliths in ferrets. However, the etiology of cystine urolithiasis in ferrets remains uncertain, and the role diet plays, if any, has not been determined.

*Feline Green Pea and Rabbit, Innovative Veterinary Diets, Pittsburgh, PA)

INFORMATION RESOURCES IN CARNIVORE NUTRITION

Laura M. Osegueda

NC State College of Veterinary Medicine
4700 Hillsborough Steet
Raleigh, NC 27606-1499
laura_osegueda@ncsu.edu

A review of key databases including CAB Abstracts, Medline, Web of Science, and Biological Abstracts are be presented with tips for when and how to get the most out of each of these resources. Websites with full text research are reviewed. Recommendations of top research materials are also provided.

CONUNDRUM II

What is a good forage?

For both traditional livestock and exotic livestock there is a normal assumption that particular forages are high-quality while others are medium- and low-quality. While this may be true on a fiber basis, could these high-quality forages potentially cause problems in other areas? For example ... too much alfalfa hay can lead to obesity concerns as well as what is commonly referred to in horses as “tying up” (too much calcium which interferes with the parathyroid gland and causes metabolic disorders).

Are there better ways to define a good forage, and if so, where do we start?

DEVELOPMENT OF A HIGH-FIBRE BAKED SUPPLEMENT FOR TORONTO ZOO PRIMATES

Adam Craig* M.Sc.^{1,2}, Marilyn Jankevicius M.Sc.², Michelle Shaw M.Sc.², Dave Barney Ph.D.^{1,2}, Jim Atkinson Ph.D.¹

¹Department of Animal & Poultry Science, University of Guelph, Guelph, Ontario, Canada
²Toronto Zoo, Scarborough, Ontario, Canada

There is mounting concern over the higher levels of easily fermentable carbohydrates such as sucrose and lower levels of fibrous pulp and seeds in many of the commercial fruits and vegetables fed to captive primates compared to wild varieties. These differences may be contributing to occurrences of gastrointestinal disorders often seen in captive primates. The overall objective of this experiment was to develop a high-fibre baked supplement in the form of a muffin designed for the primates at the Toronto Zoo.

The first part of this experiment was designed to aid in the determination of an optimal fibre source for this supplement. Psyllium, inulin, bran, and purified cellulose were four fibre sources tested in a 4x4 latin square design. Each animal was randomly assigned to one of four groups. Over eight weeks, each group was rotated through the four fibre sources for two week periods. Fecal consistency was assessed during the last two days in each period with gorillas (n=4) and orangutans (n=4).

No significant differences ($p = 0.05$) were seen between the four fibre sources tested in terms of fecal consistency for part I of the trial. Since all four fibre sources were appropriate for promoting ideal fecal consistency, the choice of which fibre source to use for the development of a primate fibre supplement was based on other health benefits of the respective fibre sources. Insoluble fibre has been shown to be very efficient as a bulking agent for the gastrointestinal tract contents but has very few additional health benefits. Soluble fibre has additional health benefits such as plasma cholesterol-lowering effects and the potential to reduce the risk for specific types of cancer. Certain sources of fibre also possess the unique characteristic of forming a viscous material as it passes through the gastrointestinal tract. An additional benefit of this unique property is the ability to prolong the absorption of glucose, helping to reduce blood glucose spikes. Cellulose is a fibre source that is almost entirely made up of insoluble fibre. Bran fibre is predominately insoluble fibre with a smaller soluble fraction. Both inulin and psyllium are predominately soluble fibre, which supplies these fibre sources with the additional health benefits mentioned above. Psyllium also has the unique characteristic of forming a viscous gel as it passes through the gastrointestinal tract. Based on this knowledge, psyllium was chosen as the fibre source for the primate fibre supplement.

The objective of the second half of the experiment was to determine the appropriate level of psyllium for use in the supplement. For this, a 3x3 latin square design was used. Each animal was randomly assigned to one of three groups. Over six weeks, each group was rotated through the three fibre levels for two week periods. Fecal consistency was evaluated on the last two days of each period with gorillas (n=5), and orangutans (n=5). The scale ranged from 1 to 5, with 1 being dry and hard and 5 representing obvious diarrhea.

All 3 levels of psyllium promoted an ideal fecal consistency (overall mean score 2.10). Based on the suggested dosages from the literature identifying the overall health benefits of dietary fibre, it is recommended that the highest level of 15g of psyllium per muffin be used in the final formulation. Increasing the overall dietary fibre levels for the Toronto Zoo primates should not only optimize gastrointestinal tract health but also result in fewer health problems overall.

DIET ASSESSMENT AND BLOOD PARAMETERS IN CAPTIVE EXOTIC EQUIDS

Michael L. Schlegel, PhD^{1*}, Michelle Shaw, MSc², Graham Crawshaw, DVM², Dave Barney, PhD², Michele Miller, DVM, PhD³, Eduardo V. Valdes, PhD³

¹Department of Animal Sciences, University of Florida, PO Box 110910, Gainesville, FL 32611 USA; ²Toronto Zoo, Scarborough, Ontario, Canada M1B 5K7; ³Disney's Animal Kingdom, PO Box 10000, Lake Buena Vista, FL 32830 USA
schlegel@animal.ufl.edu

Until recently, there has been little documentation and evaluation of diets fed to exotic equids in captivity. Edwards (2004) documented the diet nutrient content and serum mineral profiles of eight species of exotic horses, asses, and zebras at the Zoological Society of San Diego. Enteroliths (Decker et al., 1975; McDuffee et al., 1994), muscular dystrophy (Higginson et al., 1973), and laminitis (Klaus-Dieter et al., 2001) have been associated with diet, dietary imbalances, or deficiencies in captive exotic equids; therefore, evaluating diets is critical in the preventive health programs at zoological institutions. The objective of this summary was to evaluate the diets and blood mineral and vitamin concentrations of captive-housed exotic equids at Disney's Animal Kingdom and Disney's Animal Kingdom Lodge (DAK), and the Toronto Zoo (TZ).

Methods

Blood samples were collected from miniature donkeys (*Equus asinus asinus miniature*, DAK), Grant's zebras (*Equus burchellii boehmi*, DAK), Przewalski's horses (*Equus przewalskii*, TZ) and Grevy's zebras (*Equus grevyi*, TZ) from 10-Feb-1993 to 19-May-2004 during animal examinations. Typical diets fed to each species are provided in Table 1. Blood was collected by venipuncture in acid-washed or Li-heparin evacuated tubes (Becton, Dickinson and Company; Franklin Lakes, NJ 07417). Serum or plasma was harvested and frozen (-70°C at DAK; -20°C at TZ). Samples collected at DAK were analyzed within six months of collection. Banked serum samples from TZ were analyzed in 2004.

Serum samples were analyzed for mineral concentrations by inductively coupled plasma emission spectrophotometer (Ca, Cu, Fe, Mg, P, K, Na, Zn; Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI 48909 and Prairie Diagnostic Services at the University of Saskatchewan, Saskatoon SK S7N 5B4) or chemistry analyzer (Ca, P; Toronto Zoo, Scarborough, Ontario, Canada M1B 5K7), and plasma (DAK) and serum (TZ) samples were analyzed for a-tocopherol and retinol concentrations by HPLC (Animal Health Diagnostic Laboratory, Michigan State University, Lansing, MI 48909; Wildlife Conservations Society, Department of Nutrition, Bronx, New York 10460 and Prairie Diagnostic Services at the University of Saskatchewan, Saskatoon SK S7N 5B4). Multiple samples from an individual animal were averaged. Due to potential loss of vitamin activity during storage, vitamin concentrations from Przewalski's horses and Grevy's zebras are presented as averages from samples collected prior to 2004 and during 2004.

Diet evaluation

All diets (Table 1) provide adequate protein and mineral concentrations recommended for domestic horses at maintenance (NRC, 1989). Additional nutrients may need to be added for growth, reproduction, and lactation. Of the five diets in Table 1, only the miniature donkey diet does not meet their energy requirement as calculated for horses by the NRC (1989). These animals have maintained their body weight (112 kg, range: 105-118 kg) for more than two years on this diet. Guerouali et al. (2003) determined that a donkey's resting metabolism is 20% lower than that of a horse. Based on this information, the 3.94 Mcal/d provided meets the donkey's energy requirement assuming the requirement is 20% less (3.81 Mcal/d) than calculated using the horse equation. Additional chelated minerals were added to the donkey diet in 2003 to improve hoof condition and explains the high dietary Cu and Zn concentration. Both of these minerals are below what is considered toxic for horses (NRC, 1989).

Diets were recently revised for the Grant's zebras at DAK to reduce starch content of the diet, improve grass hay consumption, and decrease the risk of metabolic diseases. In addition to the diets provided (pellets and hay), the Grant's zebras had free choice access to bermudagrass (*Cynodon dactylon*)-bahiagrass (*Paspalum notatum*) pastures. Hoffman et al. (2001) discuss the need to evaluate hydrolyzable carbohydrates (CHO-H) in grains and rapidly fermentable carbohydrates (CHO-Fr) in pastures and their potential relationship to digestive and metabolic disorders in horses. The concern arises when these carbohydrates fluctuate drastically and the horse's digestive tract may not be adapted to the high CHO-H or CHO-Fr it consumes. If an animal goes off feed or is removed from the pasture, the introduction of high concentrations of CHO-H or CHO-Fr may result in a digestive disorder. Klaus-Dieter et al. (2001) described laminitis in three Przewalski's horses kept in a German semipreserve attributed to large amounts of feed high in carbohydrate (fructans) in the form of rich pasture. Pastures at DAK have ranged from 3.1 – 12.6% nonstructural carbohydrates (NSC = 100 – protein - fat - ash - NDF), 0.3 – 4.1% starch (CHO-H), and 1.3-11.8% CHO-Fr (CHO-Fr = NSC – CHO-H; Hoffman et al., 2001).

Blood parameters

All serum mineral concentrations for the four equid species are within reference ranges for domestic horses (Puls, 1994a) or each species respective ISIS (1999) reference values (Tables 2 – 5). With respect to the microminerals (Cu and Zn), not included in the ISIS (1999) reference values, the concentrations determined in the current summary are very similar to the ranges found for the Grant's zebras, Przewalski's horses, and Grevy's zebras documented by Edwards (2004).

Vitamin E (a-tocopherol) concentrations of the miniature donkeys (Table 2) and Grant's zebras (Table 3) are within the range expected in domestic horses (Puls, 1994b), and the Grant's zebras' concentrations are above those found for other captive zebras (1.5 µg/ml; Brush and Anderson, 1986). The most recent blood samples collected from the Przewalski's horses, and Grevy's zebras have a-tocopherol concentrations 25 to 30% below the reference ranges, respectively. It is also evident that there may have been vitamin E degradation during storage in samples collected before 2004 (Table 4 and 5).

The NRC (1989) recommends at least 50 IU vitamin E/kg DM for maintenance and 80-100 IU/kg DM for foals, pregnant and lactating mares, and working horses. Based on these recommendations, only the previous diet used for the Grant's zebras is adequate and in part explains why these equids had the highest blood concentrations. The Grant's zebras also have daily access to pasture year round which may provide additional vitamin E not included in the diet nutrient concentrations listed in Table 1. Although the donkeys' plasma α -tocopherol concentrations are within reference ranges, their diet may be marginal in vitamin E. As for the Przewalski's horses and Grevy's zebras, the low α -tocopherol concentrations observed may be a result of marginal dietary vitamin E or loss of α -tocopherol activity in serum during storage. Additionally, serum vitamin E concentration of the Przewalski's horses in the current study are greater than those found in Przewalski's horses in southeast England zoos (1.12 $\mu\text{g/ml}$; Brush and Anderson, 1986), but significantly lower than concentrations found in semi-free-ranging adult Przewalski's horses in the Ukraine (6.58 $\mu\text{g/ml}$; Dierenfeld et al., 1997).

Retinol concentrations for the Przewalski's horses (samples collected in 2004, Table 4) are within the reference range for domestic horses (Puls, 1994b). The miniature donkeys (Table 2) and Grant's zebra (Table 3) had retinol concentrations below or just within reference ranges. (Puls, 1994b). Based on a requirement of 1830 IU vitamin A/kg DM (NRC, 1989), the donkeys are receiving an inadequate amount of vitamin A. The vitamin A content of the donkey diet can be improved through the addition of a small amount of pellet to supply the additional vitamin A while maintaining the body weight of the animals. Although the current Grant's zebra diet is below the recommended vitamin A concentration, the Grant's zebras have access to pasture, which is an abundant source of vitamin A precursors (NRC, 1989). The Grevy's zebra diet was the same as the Przewalski's horse diet and there were insufficient recent serum retinol values for comparison, but it is reasonable to expect the Grevy's zebra to have adequate vitamin A status similar to the Przewalski's horses.

Summary

Although some of the α -tocopherol and retinol concentrations were below the reference ranges, no deficiency symptoms have been observed at any institution. Health monitoring and feed evaluation is an ongoing process with diet revisions undertaken as new information and data are obtained. These data provide a baseline for future evaluation of the mineral-vitamin status and diets of exotic equids in captivity.

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Table 1. Body weight ranges, diet composition, and selected nutrient concentrations of captive exotic equid diets.

Item	Miniature donkey	Grant's zebra		Przewalski's horse	Grevy's zebra
		Previous	Current		
Body wt., kg	112	301		293	367
DE req., Mcal/d ^a	4.76	10.43		10.19	12.41
Diet ingredient	Daily diet offered, kg as-fed				
Pellets	-	2.00	0.60	-	1.20
Bermudagrass hay	1.36	4.80	-	-	-
Timothy hay	-	-	8.20 ^b	-	7.00
Electrolytes ^c	0.325	-	-	-	-
Enrichment items	0.02 ^d	Variable ^e	Variable ^e	-	-
Training items	-	Variable ^{e,f}	Variable ^{e,f}	-	0.30 ^d
Browse or pasture	-	Variable ^e	Variable ^e	-	Variable ^e
Chelated minerals ^g	0.01	-	-	-	-
White and trace mineral salt	-	Free choice ^e	Free choice ^e	-	Free choice ^e
Cobalt iodized salt	-	-	-	-	Free choice ^e
Nutrient	Concentration, DM basis				
DM, %	91.22	89.63	90.56	-	86.42
CP, %	8.02	12.52	9.47	-	10.74
ADF, %	28.24	33.17	35.73	-	31.66
Crude fat, %	0.58	1.85	1.35	-	2.75
DE, Mcal/kg ^h	2.52	2.25	2.10	-	2.33
DE, Mcal/d ⁱ	3.94	13.71	16.74	-	17.12
Ca, %	0.27	0.57	0.35	-	0.55
Cu, µg/g	65	15	18	-	29
Fe, µg/g	71	106	131	-	234
Mg, %	0.12	0.21	0.15	-	0.16
P, %	0.17	0.35	0.26	-	0.28
K, %	1.84	1.63	2.02	-	1.74
Na, %	0.89	0.15	0.08	-	0.05
Zn, µg/g	184	72	40	-	100
Ca:P ratio	1.59	1.63	1.35	-	1.96
Vitamin A, IU/kg	1300	3950	2370	-	24162
Vitamin E, IU/kg	42.35	136.5	26.48	-	34.43

^aDE required = 1.4 + 0.03 x (BW, kg), (NRC, 1989, adapted from Pagan and Hintz, 1986).

^bAdditional quantities are offered on exhibit, but consumption is variable dependent on environmental factors.

^cPerform 'N Win, Buckeye Nutrition, Dalton, OH 44618.

^dRaw apples and carrots.

^eNot included in nutrient concentrations below.

^f200 g raw carrot, 28.6 g shredded citrus pulp, 7.1 g herbivore gel, and 21.4 shredded beet pulp.

^g4-Plex, Zinpro Corporation, Eden Prairie, Minnesota 55344.

^hCalculated DE (Mcal/kg) = 4.07 - 0.055 x (ADF, %); NRC, 1989.

ⁱDaily DE offered (Mcal) = Daily diet offered (kg, as-fed) x DM% x dietary DE (Mcal/kg)

Table 2. Serum mineral and plasma vitamin concentration of miniature donkeys (*Equus asinus asinus miniature*).

Mineral	n ^a	Mean	SD	Range		Reference values	
				Low	High	ISIS, 1999 ^b	Puls, 1994
Ca, mg/dl	2	12.3	4.8	12.0	12.7	9.3-14.3	10.0-13.0 ^d
Cu, µg/dl	2	90	6	85	94	NA ^c	50-200 ^d
Fe, µg/dl	2	118	3.2	116	120	78-215	84-257 ^d
Mg, mg/dl	2	2.25	0.175	2.12	2.37	1.30-2.70	1.8-3.5 ^d
P, mg/dl	2	3.29	0.156	3.18	3.40	1.8-11.0	2.7-5.0 ^d
K, mEq/l	2	3.15	0.12	3.06	3.24	3.4-5.6	2.40-4.09 ^d
Na, mEq/l	2	133	2.2	131	134	128-145	130-143 ^d
Zn, µg/dl	2	53	3.6	50.7	55.7	NA	50-150 ^d
Ca:P ratio	2	3.8	0.32	3.6	4.0	NA	NA
a-Tocopherol, µg/ml	2	2.54	0.608	2.11	2.97	NA	2.00-10.00 ^e
Retinol, µg/ml	2	0.169	0.046	0.136	0.201	NA	0.175-0.350 ^e

^aTwo animals, each with four, three, and two samples for mineral, a-tocopherol, and retinol analysis, respectively, collected between 1999 and 2004.

^bAfrican wild ass (*Equus asinus*), both sexes, all ages combined.

^cNot available.

^dPuls, 1994a.

^ePuls, 1994b.

Table 3. Serum mineral and plasma vitamin concentration of Grant's zebra (*Equus burchellii boehmi*).

Mineral	n ^a	Mean	SD	Range		Reference values	
				Low	High	ISIS, 1999 ^b	Puls, 1994
Ca, mg/dl	14	10.4	0.39	9.7	1.10	8.2-13.5	10.0-13.0 ^d
Cu, µg/dl	14	97	13.9	61	123	NA ^c	50-200 ^d
Fe, µg/dl	14	124	30.4	82	190	81-111	84-257 ^d
Mg, mg/dl	14	1.50	0.143	1.25	1.88	0.61-2.20	1.8-3.5 ^d
P, mg/dl	14	4.83	0.8	3.47	6.4	2.3-11.2	2.7-5.0 ^d
K, mEq/l	14	2.65	0.10	12.5	18.8	2.1-7.2	2.40-4.09 ^d
Na, mEq/l	14	139	4.88	133	150	128-149	130-143 ^d
Zn, mg/dl	14	70	12.9	45	93	NA	50-150 ^d
Ca:P ratio	14	2.2	0.3	1.6	3.0	NA	NA
a-Tocopherol, µg/ml	13	4.27	1.40	2.17	7.48	NA	2.00-10.00 ^e
Retinol, µg/ml	13	0.175	0.055	0.073	0.297	NA	0.175-0.350 ^e

^aTwenty-five samples from 14 individuals for mineral analysis, 30 samples from 13 individuals for a-tocopherol analysis, and 24 samples from 13 individuals for retinol analysis.

^bCommon zebra (*Equus burchellii*), both sexes, all ages combined.

^cNot available.

^dPuls, 1994a.

^ePuls, 1994b.

Table 4. Serum mineral and vitamin concentration of Przewalski's horse (*Equus przewalskii*).

Mineral	n ^a	Mean	SD	Range		Reference values	
				Low	High	ISIS, 1999 ^b	Puls, 1994
Ca, mg/dl	11	10.4	1.28	8.6	13.2	9.4-12.9	10.0-13.0 ^d
Cu, µg/dl	12	154	40	99	256	NA ^c	50-200 ^d
Fe, µg/dl	12	169	46	63	256	46-410	84-257 ^d
Mg, mg/dl	12	1.40	0.114	1.25	1.57	0.90-2.02	1.8-3.5 ^d
P, mg/dl	11	3.97	0.83	2.94	5.82	0-6.8	2.7-5.0 ^d
K, mEq/l	12	4.25	0.43	3.48	4.93	3.1-6.8	2.40-4.09 ^d
Na, mEq/l	12	140	3.2	135	147	120-162	130-143 ^d
Zn, µg/dl	12	103	31	53	153	NA	50-150 ^d
Ca:P ratio	11	2.80	0.59	1.88	3.70	NA	NA
a-Tocopherol, µg/ml							
prior to 2004	10	0.65	0.633	0.13	1.82	NA	2.00-10.00 ^e
during 2004	5	1.50	0.660	1.03	2.66		
Retinol, µg/ml							
prior to 2004	10	0.202	0.055	0.130	0.310	NA	0.175-0.350 ^e
during 2004	5	0.254	0.070	0.170	0.360		

^aSeventeen samples from 12 individuals.

^bPrzewalski's horse, both sexes, all ages combined.

^cNot available.

^dPuls, 1994a.

^ePuls, 1994b.

Table 5. Serum mineral and vitamin concentration of Grevy's zebras (*Equus grevyi*).

Mineral	n ^a	Mean	SD	Range		Reference values	
				Low	High	ISIS, 1999 ^b	Puls, 1994
Ca, mg/dl	9	9.5	1.78	7.7	12.0	8.8-12.8	10.0-13.0 ^d
Cu, µg/dl	9	124	25	95	174	NA ^c	50-200 ^d
Fe, µg/dl	9	160	24	125	206	27-239	84-257 ^d
Mg, mg/dl	9	1.33	0.14	1.12	1.53	1.07-3.10	1.8-3.5 ^d
P, mg/dl	9	3.96	0.821	2.726	5.174	2.6-9.9	2.7-5.0 ^d
K, mEq/l	9	3.62	0.30	3.18	4.21	3.0-5.7	2.40-4.09 ^d
Na, mEq/l	9	142	2.3	138	145	125-157	130-143 ^d
Zn, µg/dl	9	103	36	72	191	NA	50-150 ^d
Ca:P ratio	9	2.64	0.64	1.84	3.76	NA	NA
a-Tocopherol, µg/ml							
prior to 2004 ^f	8	0.479	0.366	0.15	1.30	NA	2.00-10.00 ^e
during 2004	1	1.41					
Retinol, µg/ml							
prior to 2004	9	0.156	0.040	0.100	0.220	NA	0.175-0.350 ^e
during 2004	1	0.140					

^aFourteen samples from 9 individuals.

^bGrevy's zebra, both sexes, all ages combined.

^cNot available.

^dPuls, 1994a.

^ePuls, 1994b.

^fTwo samples from one individual contained a-tocopherol concentrations below the detectable limit (0.1 µg/ml).

DOES VARIATION IN DIGESTION AMONG INDIVIDUALS PLAY A ROLE IN NUTRITIONAL HEALTH OF THE COMMON MARMOSET?

E. Wilson Myers^{1*} and Michael L. Power², PhD

¹ Colorado College, Colorado Springs CO

² Smithsonian's National Zoological Park, Washington DC 20008, powerm@nzp.si.edu

Common marmosets (*Callithrix jacchus*) are small (about 350g), New World monkeys that belong to the monophyletic primate family Callitrichidae. All callitrichids are omnivorous, and feed on fruit, gum, other plant exudates, nectar, invertebrates and small vertebrates. Despite their long history of captive management, nutrient requirements for the common marmoset are poorly characterized. Their nutritional husbandry has been informed more by practical and anecdotal experience than it has been by nutritional science. The few well-designed nutritional studies on healthy common marmosets have generally found that nutrient requirements are not appreciably different from that of other anthropoid primates. However, data from other research have suggested that marmosets have higher requirements for protein, energy, and vitamin D.

Nutrition and dietary husbandry of marmoset colonies is a major concern among colony managers in the United States. Marmosets are known to suffer from diarrheas and from inflammation of the intestinal tract. These conditions are likely related, and both can contribute to poor nutrient absorption. Marmoset colony managers have routinely found that, regardless of the nutrient levels in the diet, they often have "poor doers" in their colony. These animals generally have recurrent or chronic diarrhea, lose weight, and eventually die. The term "Wasting Marmoset Syndrome" has been applied to the end stages of this pathology.

We propose that much of the confusion over nutrient requirements in common marmosets, and the existence of "poor doers" in colonies, stems from the susceptibility of the common marmoset to inflammation of the upper intestinal tract which results in malabsorption of nutrients. Animals with differing levels of intestinal inflammation should exhibit differing abilities to digest identical diets. We examined digestion in a colony of common marmosets fed a single-item, purified diet to examine the extent of individual variation in digestion.

Methods

Subjects: The animals used in this study were 13 singly-housed adult common marmosets (5 female and 8 male) housed at the Southwest National Primate Research Center in San Antonio TX. Animals ranged from 1 year and 10 months old to 5 years and 2 months old. A common marmoset is sexually mature at 18 months, and reaches its full adult size by three years of age.

Diet: A single-item, purified agar-gelled diet was given to all subjects. This diet is the base colony diet, and all animals had been fed it from birth.

Digestion trial protocol: Two four-day-long digestion trials were conducted on consecutive weeks. Animals were given liquid-phase (cobalt EDTA) and solid-phase (chromium mordanted fiber) at the beginning of each trial. All amounts of food offered to the animals each day were

weighed, and a fresh sample of the offered food was frozen for later analyses. All uneaten food and feces were collected, and stored frozen until assayed for dry matter, energy and nitrogen.

Laboratory analyses: Assays were completed at the Nutrition Laboratory of the Smithsonian's National Zoological Park, Washington DC. All food samples and feces were freeze-dried and then oven dried at 100°C, weighed, and then ground for subsampling. Refusals were oven dried at 100°C, weighed, and then discarded. Energy was determined using an adiabatic bomb calorimeter and nitrogen was determined using an elemental analyzer. Crude protein was calculated as nitrogen X 6.38, as the protein source was lactalbumin, a milk protein.

Calculation of digestive parameters: Coefficients of apparent digestibility of dry matter (ADMD), energy (ADE) and crude protein (ADCP): The amount of nutrient in ingested food minus the amount of nutrient in collected feces, all divided by the amount of nutrient in the ingested food.

Results

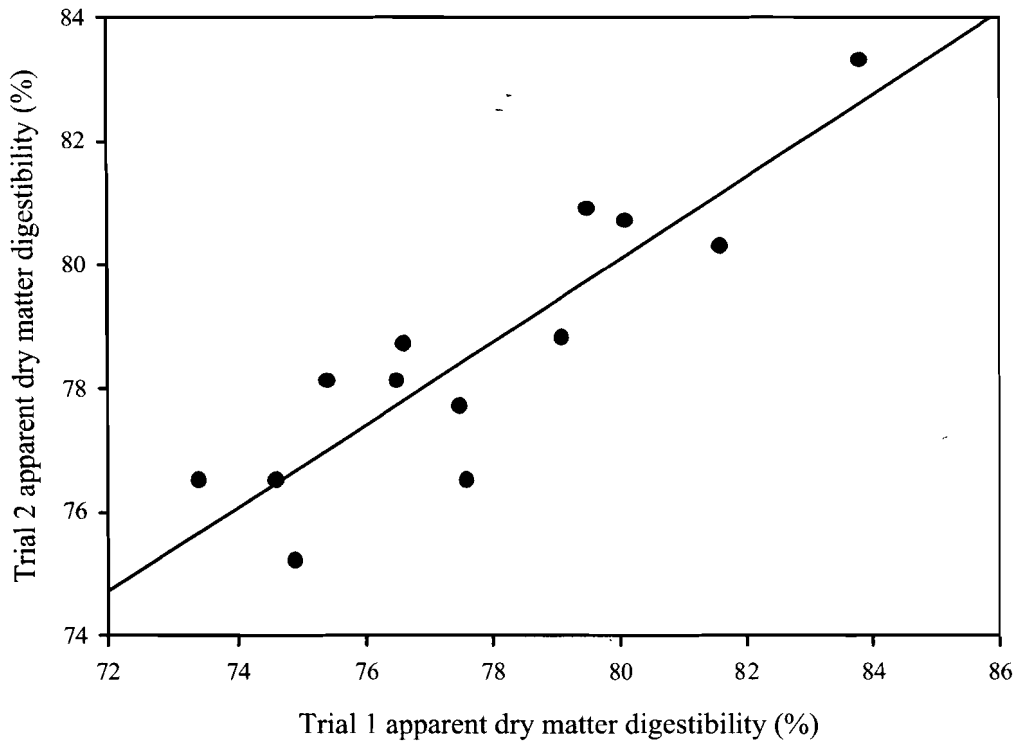
Mean DMI was 13.3 ± 0.8 g/day, for an estimated gross energy intake (GE) of 57.1 ± 3.5 kcal/day. Mean ADDM was $78.2 \pm 0.7\%$, mean ADE was $79.0 \pm 1.0\%$, and mean ADCP was $63.3 \pm 2.4\%$. Neither food intake nor ADDM varied significantly between the two trials, though the values tended to be higher in trial 2 ($p=.083$ and $p=.060$, respectively). Within animals food intake and ADDM were highly correlated between trials ($r=.827$ and $r=.884$, respectively, $p<.001$; see Figure 1 for ADDM data). Mean values for ADDM among individuals ranged from 74.9% to 83.5%. The variation in ADE was greater, 74.0% to 86.4%, and even greater in ADCP (53.4% to 79.8%). This variation in apparent digestibility was not related to mean transit time nor to body weight (data not presented).

Discussion

There was significant variation among individuals in the ability to digest a single-item, purified diet. All these animals were born into the colony, and had been eating this diet (though not as the sole food) since weaning. The variation in apparent digestibility was not related to variation in mean transit time or body weight among individuals. We interpret this to mean that the variation in apparent digestibility is possibly a direct reflection of variation in absorptive capabilities of the intestinal tracts among individuals.

Currently, further digestion trials are underway on older animals, and on pregnant females. In addition, blood samples have been taken from individuals involved in the digestion trials for serum chemistries and for determination of serum 25-OH-vitamin D concentration. It is a common finding in this, and other marmoset colonies, that serum concentration of 25-OH-vitamin D is highly variable among individuals, even when, as in this colony, animals are on completely identical diets. We hypothesize that this variation is directly correlated with the extent of intestinal inflammation and hence absorptive capacity of the gut. We hypothesize that 25-OH-vitamin D concentrations will be correlated with apparent digestibility. We also plan on examining serum markers of systemic inflammation, and hypothesize that these will be negatively correlated with apparent digestibility.

Figure 1. The apparent dry matter digestibility (ADDM) of the diet varied among animals, however, the values for ADDM for each animal were consistent between the two trials.



DIET INTAKE OF CARMINE BEE-EATERS (*MEROPS NUBICUS NUBICUS*) AT DISNEY'S ANIMAL KINGDOM

Alejandra Renjifo, BS^{1*}, Heather Blum, BS¹, Eduardo V. Valdes, PhD^{1,2,3}

¹Disney's Animal Kingdom, PO Box 10,000, Lake Buena Vista, FL; ²University of Guelph, Department of Animal and Poultry Science, Ontario, Canada, N1G 2W1; ³University of Central Florida, 4,000 Central FL. Blvd., Orlando, Florida
alejandra.renjifo@disney.com

The Carmine bee-eater (*Merops nubicus nubicus*) resides in the African tropical savannah. Its diet consists almost solely of airborne insects (Fry, 1984). At Disney's Animal Kingdom, an insect, meat, pellet, vegetable diet is offered. This study was designed to attain information on item preference, assure consumption of meat, and reduce waste.

Methods

Two three-day intake trials were conducted at Disney's Animal Kingdom's (DAK) Avian Research Center on an adult group (4.2, average body weight = 47.5 g) of the insectivorous carmine bee-eaters on February 19-21 and March 6-8, 2002. Feeding took place on an elevated platform and other birds in the enclosure (6.1 x 9.1 x 2.4 meters) were removed for the duration of the study. Insects were offered on the feeding platform and air-tossed by the keeper staff. Diets were prepared fresh daily. Half was fed in the morning after preparation, and the remainder fed in the early afternoon.

The diet offered (Table 1) consisted of Mazuri Parrot Breeder[®] (PB; PMI Nutrition International, St. Louis, MO 63166), Zeigler's Bird of Paradise[®] (BOP; Zeigler Bros, Inc., Gardners, PA 17324), Hill's Science Diet - Adult Dog Food (Hill's Pet Nutrition, Inc., Topeka, KS 66601), carrots, crickets (*Acheta domesticus*), superworms (*Zophobas morio*), wax worms (*Galleria mellonella*), and meat mix. The meat mix was composed of Toronto Zoo Carnivore Diet (Milliken Meat Products LTD., Scarborough, Ontario, Canada M1V 3F1), Mazuri Small Bird Breeder[®], Bevo Universal Insectivore Food and a supplement mix (2:1:1 Roxanthin Red (Sensient Color, St. Louis, Missouri 63106), Necton S (Nekton-Produkte, Tarpon Springs, FL 34689), and Emerald Nutri-Support (Lafeber Company, Cornell, IL 61319).

Dry matter and moisture loss information were determined for each diet component using control diets. Manufacturer and laboratory analyses for each item were used with the Zootrition[™] Dietary Management Software (Version 2.0, Wildlife Conservation Society, Bronx, NY) to calculate nutrients in the offered and consumed diet.

Results and Discussion

The group of six birds ate an average of 38.4 g dry matter (DM) per day resulting in an individual consumption of 6.4 g, or 13.5% of body weight. This is comparable to the 13.8% of body weight consumed by Blue-throated bee-eaters (*Merops viridas*; Nagy, 2001).

The birds displayed a clear preference for the three invertebrates and the meat mix (Table 1). The PB, BOP, dog food, and carrots were not consumed as readily. Approximately 60% of the total DM offered was refused, representing a yearly waste of 9.3 kg of food. Tables 2 and 3 illustrate the nutrient composition for the daily diet offered and consumed. The birds selected for foods that were higher in energy, protein, fat, fiber and Vitamin A; and, in general, lower in minerals than the diet offered. Their consumption for all minerals was similar or lower than what the offered diet provided. It should be noted that a 1:1 Ca:P ratio was maintained in the consumed diet. However, insect gut loading was not accounted for in the nutrient analysis of either offered or consumed diets.

Conclusion

The good health records of the birds may be an indication that the diet offered to the bee-eaters at DAK fulfills this species' maintenance requirements. Since this experiment was conducted, the BOP was deleted from the diet due to non-consumption and Bevo was replaced with Quiko Goldy (Bocholt, Germany) due to availability. To date, one pair has successfully raised one chick out of a clutch of five.

Acknowledgements

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Table 1. Food items offered and consumed as a percent of total DM in a Carmine Bee-eater (*Merops nubicus nubicus*) diet.

Food Item	% of total offered	% of total intake
Carrot	1.37	0.46
Hill's Science Diet - Adult Dog Food	16.26	9.51
Mazuri Parrot Breeder	14.00	3.02
Zeigler Bird of Paradise	11.14	2.57
Meat Mix*	37.22	51.51
Adult Crickets	2.27	3.77
Super worms	15.94	26.14
Wax worms	1.80	3.01

* Meat Mix: Toronto Zoo Carnivore Diet, Mazuri Small Bird Breeder[®], Bevo Insectivore Food and a supplement mix (Roxanthin Red , Necton S, and Emerald Nutri-Support).

Table 2. Selected nutrient concentrations of diets offered and consumed by Carmine Bee-eaters (*Merops nubicus nubicus*).

Diet Type	GE ---- kcal/g DM ----	ME	CP ----- % DM -----	Cfat	ADF	Vit A IU/g DM
Offered	4.89	3.56	30.15	16.11	3.78	68.69
Intake	5.06	3.77	34.80	21.62	3.86	72.69

Table 3. Mineral concentrations of diets offered and consumed by Carmine Bee-eaters (*Merops nubicus nubicus*).

Diet Type	Ca ----- % DM -----	P	Mg	Na	K	Cu ----- mg/kg DM -----	Fe	Mn	Zn
Offered	1.00	0.81	0.14	0.23	0.54	18.31	145	46	113
Intake	0.74	0.74	0.12	0.23	0.40	14.82	114	32	102

CONUNDRUM III

Fishes commonly absorb many minerals and electrolytes from their water. In captivity, the diets provided to fish are sometimes based on availability rather than careful evaluation of the natural history and needs of the species. In a series of cases mineralization of internal body organs (spiral valve initially, followed by other soft tissues) is occurring in juvenile and young adult cownosed rays. These marine fish are held in artificial sea water and fed a chopped thawed frozen fish mix primarily of capelin and herring. In what ways can the potential of dietary vs. water sources of the problem be evaluated?

POSSIBLE COPPER DEFICIENCY IN A HERD OF ZOO GOATS

Maryanne E. Tociidlowski, DVM, Dipl. ACZM

Houston Zoo, Inc.
1513 N. MacGregor, Houston Texas 77030
mtociidlowski@houstonzoo.org

In October of 2000, the Houston Zoo opened its new Children's zoo. This area of the zoo specializes in native Texas species as well as minor breeds in the farm section. Separate, established herds of approx 35 goats (African pygmy and Nigerian dwarfs) and 15 sheep (St. Croix, Barbados, and Jacob crosses) were commingled into the new contact yard. Nubian goats were added several months later. Approximately 1 year later, the incidence of minor upper respiratory disease, hair coat changes, lameness's and general unkemptness in the goat herd seemed to increase, whereas the animals had been previously fairly healthy. Over time, discussions ensued about differences between the two areas where the animals were housed. No conclusions were made except that the sheep and goats were now housed together in a new area. Husbandry and personnel remained similar to that in the old yards. Clinical pathology and serology testing performed each year were overall unremarkable other than anemia that seemed to run in the goat herd. In early June 2003, a discussion about diets and the different dietary needs between sheep and goat brought to light the possibility of a copper deficiency in the goats. The combined sheep and goat herd was being fed a Purina Goat and Sheep pellet as well as coastal hay and a small amount of alfalfa. Serum copper testing ensued with the goats and a few representative sheep. The goats were found to have a markedly low serum copper level (<0.1-1.45 ppm, lab normal: 0.8-1.2 PPM) whereas the sheep were in the normal range (0.55-1.22, lab normal: 0.8-1.2 PPM).

Copper deficiency, hypocuprosis, can be either primary due to lack of copper in the diet, or secondary where there is an abnormal level of molybdenum, sulfur, iron or other substances in relation to copper in the diet. Copper is required for multiple metabolic pathways such as immune system function, hemoglobin production, and mammalian enzymes. Possible signs of copper deficiency include microcytic anemia, abnormal hair growth and color, increased susceptibility to disease, enlarged joints and lameness (enzootic ataxia), GI problems as well as other syndromes. Animals with a primary deficiency appear to have more severe clinical signs, with young animals being more susceptible. Goats require more copper in the diet than sheep or cattle and deficiency seems to be magnified in pygmy and dwarf goat species. Diagnosis should be based on history, clinical signs, and blood and dietary levels. Treatment consists of correcting the source of the deficiency and possibly supplementing with copper orally or parenterally.

Once it was determined that serum copper levels in the goats were abnormal, a change in the sheep and goat feeding practices was instituted. The two species were separated and fed a species appropriate pellet. The goats were given Mazuri® ADF-16 herbivore pellet, which contains 20 PPM copper. The sheep were started on a Mazuri® Sheep Locu pellet, which contains 4.7 PPMm copper. Subsequent retesting of the goat herd for serum copper levels showed a marked improvement as little as 1 month later after changing the feeding protocol. Serum copper levels measured one year later showed the majority of goats were now in the

normal range for copper levels (see chart below). Clinical signs of upper respiratory disease, hair coat abnormalities, and general unthriftiness have abated.

Goat and Sheep Serum Copper Levels (PPM)

Species	Name	Weight (Kg)	June-03	July-04
Goat-African pygmy	Wednesday	19.0	0.12	0.63
Goat-African pygmy	Stripes	30.0	<0.1	0.54
Goat-African pygmy	Meenie	16.0	0.09	0.46
Goat-African pygmy	Baby Gold	23.0	<0.1	0.72
Goat-Nigerian dwarf	Lilly	29.0	<0.1	0.89
Goat-Nigerian dwarf	Medley	23.0	0.75	0.79
Goat-Nigerian dwarf	Ragweed	21.0	<0.1	0.91
Goat-Nigerian dwarf	Spice	22.0	<0.1	1.03
Goat-Nigerian dwarf	Sugar	29.0	<0.1	1.22
Goat-Nigerian dwarf	Thunder	47.0	0.18	0.76
Goat-Nigerian dwarf	Muke	19.0	0.10	0.68
Goat-Nigerian dwarf	Sacha	29.0	0.54	0.75
Goat-Nigerian dwarf	Butterscotch	27.0	0.23	0.78
Goat-Nigerian dwarf	Messina	29.0	0.38	1.06
Goat-Nigerian dwarf	Flash	30.0	1.45	1.13
Goat-Nigerian dwarf	Ginger	25.0	0.52	0.88
Goat-Nigerian dwarf	Maryanne	20.0	0.39	0.60
Goat-Nigerian dwarf	Professor	35.0	0.34	0.92
Goat-Nigerian dwarf	Quatro	33.0	0.39	0.85
Goat-Nigerian dwarf	Lisa Marie	21.0	0.21	0.64
Goat-Nigerian dwarf	Annabell	19.0	0.32	0.76
Goat-Nubian	Sampson	69.0	0.42	0.74
Goat-Nubian	Big Al	54.0	0.44	0.89
Goat-Nubian	Nicky	59.0	0.39	0.70
Goat-Nubian	Brownie	49.0	0.42	0.74
Goat-Nubian	Oreo	47.0	0.78	1.00
Sheep-Barbados	Nellie	25.0	0.55	
Sheep-cross	Mark	39.0	0.66	
Sheep-St Croix	Papaya	44.0	0.79	
Sheep-St Croix	Vincent	51.0	1.22	

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**PLASMA ELECTROLYTE CONCENTRATIONS IN CAPTIVE AND FREE-RANGING
AFRICAN PENGUINS (*SPHENISCUS DEMERSUS*) MAINTAINED WITH AND
WITHOUT DIETARY SALT SUPPLEMENTS**

Lisa M. Mazzaro, PhD¹, Allison Tuttle, DVM^{1,5*}, Jeff Wyatt, DVM², Jeremy Goodman,
DVM³, Edmund Kadyszewski, MS⁴, and J. Lawrence Dunn, VMD¹

1: Mystic Aquarium, Mystic, Connecticut 06355-1997; 2: Seneca Park Zoo, Rochester, NY
14621-1096; 3: Potawatomi Zoo, South Bend, IN 46615; 4: Pfizer, Groton, CT;
5: North Carolina State University, College of Veterinary Medicine, Raleigh, NC 27606
email: allison_tuttle@ncsu.edu; lmazzaro@mysticaquarium.org

African penguins, *Spheniscus demersus*, are common display animals in North American zoos and aquariums. At present 43 American Zoo and Aquarium Association (AZA) accredited institutions maintain over 700 of these birds. A survey of facilities found that only seven institutions maintain their birds in “saltwater habitats” (natural, brine or artificial seawater systems) whereas the remaining institutions provide freshwater habitats. Penguins maintained in freshwater habitats commonly receive dietary salt supplements, though evidence is lacking as to the value of this practice.

This study was designed to evaluate the necessity for salt supplementation in African penguins. We report results of a randomized, ten month comparison of plasma electrolytes (Na, Cl and K) between groups of 10 salt supplemented and non-supplemented birds living in a freshwater environment. The results showed no significant differences between the two groups. An inter-facility comparison at the same timepoints revealed temporal pattern differences in analyte values between the facilities, however, the absolute concentrations did not exceed clinical ranges found in healthy birds. Furthermore, single time point comparisons between wild and captive African penguins indicate similar sodium concentrations, while potassium and chloride concentrations varied between groups. Finally, plasma electrolyte levels of twenty birds remained statistically unchanged after a sixty day withdrawal from salt supplementation.

This study provides experimental evidence that African penguins maintained in freshwater exhibits, on a herring, capelin and squid based diet, do not require salt supplementation. In addition to the practical implications regarding the need for salt supplementation for captive birds, the results have theoretical significance as well. They provide evidence that penguins do not require the ingestion of high salt concentrations to remain in electrolyte balance and thus that penguin ion regulatory mechanisms are in that sense, similar to those of terrestrial mammals and birds.

CALCIUM SUPPLEMENTATION IN DESERT GRASSLAND WHIPTAILS (*CNEMIDOPHORUS UNIPARENS*)

Robert MacLean, DVM,* Barbara Wolfe, DVM, PhD, Dipl. ACZM

North Carolina Zoological Park, 4401 Zoo Pkwy, Ashboro, NC 27205
ramaclea@ncsu.edu, barbara.wolfe@ncmail.net

The North Carolina Zoological Park (NCZ) maintains a colony of desert grassland whiptail lizards (*Cnemidophorus* .) (DGW). These small reptiles are found in the arid and semiarid grasslands and desert scrub of the Southwest in North America and in Mexico. They are interesting animals, particularly in the fact that they reproduce by parthenogenesis: the females lay eggs that are clones of themselves and their ancestors. In the wild, these lizards continue to reproduce successfully, generation after generation. In captivity, however, colonies experience lineage senescence, where a particular generation, usually on or before F₅, will fail to thrive or reproduce, thus limiting the number of successive generations a colony can produce.¹ It is likely that the causative factor for lineage senescence lies with the husbandry of these animals, either in the form of a missing factor (e.g., a nutrient), an added factor (e.g., a toxin), or a combination of both.ⁱ

In 1998 the DGW colony at the NCZ was experiencing an increase in mortality at or near the time of oviposition associated with dystocia, egg yolk coelomitis, seizing, and/or vertebral fractures that was presumed to originate from a calcium/phosphorus (Ca/P) imbalance and/or other nutrient deficiencies such as vitamin D₃. The colony was housed in small glass aquariums with unscreened UVA/B (Sylvania 350) and bright fluorescent light sources. The diet then consisted primarily of crickets fed a commercial cricket diet with a calcium content of 8% dry matter (DM) and dusted with a mixture of oyster shell and cornstarch. A nutritionist was consulted who suggested the possibility of a broad range of nutritional deficiencies in the DGW colony. Recommendations included changing the dust to a fine calcium carbonate as well as another dust to be used on alternate days that contained nutrients such as vitamin D₃ and other vitamins as well as trace minerals. The cricket diet was also modified to produce healthier crickets by switching to an avian maintenance feed with a reduced calcium content of approximately 1% DM.

Soon after the above husbandry changes, a controlled trial was initiated to investigate the potential benefits of calcium supplementation prior to oviposition. Animals were separated into either a control group (group 1) or a treatment group that received oral calcium supplementation (group 2). Supplementation with 1.2mg calcium glubionate (Calciquid, Breckenridge Pharmaceutical, Inc., Boca Raton, FL) PO was initiated for group 2 by keepers at the first sign an animal appeared gravid. Treatment was continued daily until oviposition or a maximum of 14d. At oviposition or the final treatment day, a blood sample was collected for Ca and P analysis. Neither ionized Ca nor albumin nor other parameters were analyzed due to the small sample volumes. Over a 4 month period 17 control and 16 treatment animals were accumulated.

No significant differences were detectable in the total Ca, P, or Ca/P, and oral Ca supplementation of gravid animals was discontinued. The occurrence, however, of egg yolk coelomitis, seizures, and vertebral fractures has decreased since the above husbandry changes were implemented (Fig 1). The occurrence of dystocia has remained unchanged. These data are expressed as a percentage of lesions noted on necropsy reports to control for a fluctuating colony size. It is likely that the above husbandry changes have been beneficial to this colony as no other significant changes to husbandry or environment are known at this time. This colony of DGW is exhibiting signs of lineage senescence, and its population has declined in recent years. Currently, however, there are F₇ generation individuals that appear healthy and may reproduce. Although the husbandry has significantly improved, we have yet to simulate all essential factors present in the natural environment of these animals.

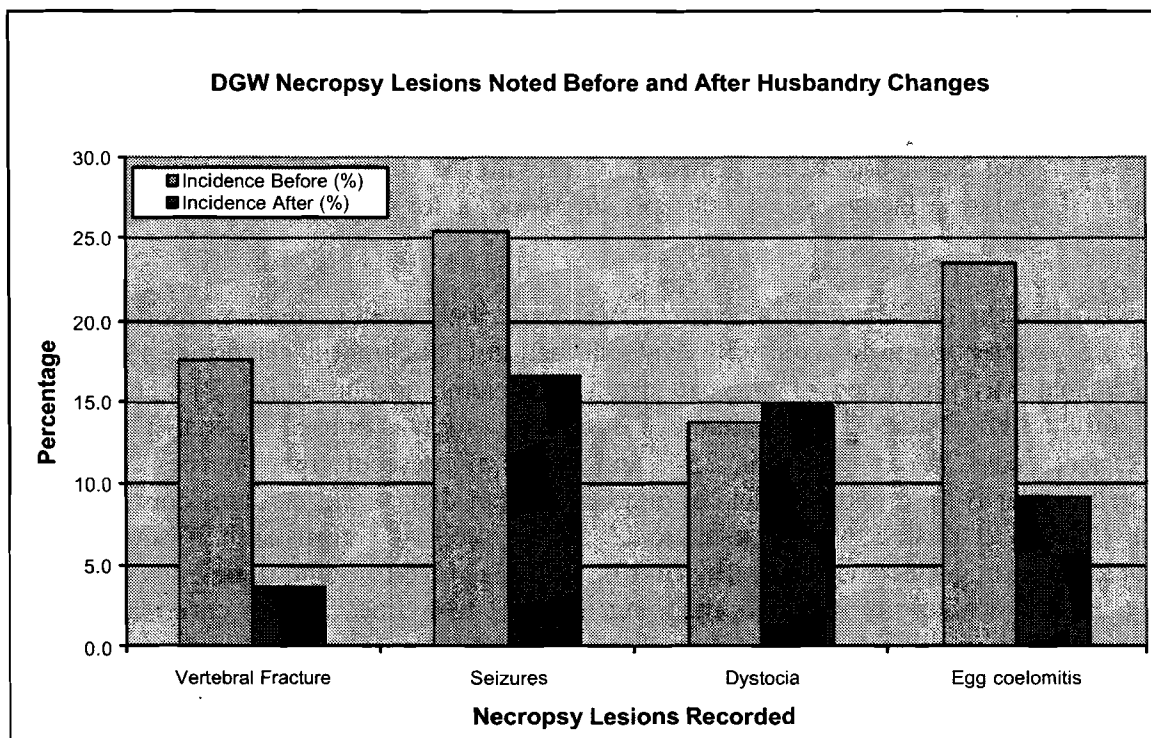


Figure 1. Occurrence of vertebral fracture, dystocia, and egg coelomitis lesions as well as historical seizures noted on necropsy reports of DGW both before (n=51 reports, 4/26/96 to 4/30/99) and after (n=54 reports, 5/1/99 to 10/9/04) husbandry changes expressed as a percentage of total reports. Only animals >4mo of age are included. Known iatrogenic mortalities (e.g., surgical) are excluded.

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ANALYTICAL SERVICE CENTERS IN COLLEGE OF AGRICULTURE AND LIFE SCIENCES AT NCSU, RALEIGH, NC, USA

John Classen¹, Wayne P. Robarge^{2*}, and Damian Shea³

¹Department of Biological and Agricultural Engineering

Email: John_Classen@ncsu.edu

²Department of Soil Science

Email: Wayne_Robarge@ncsu.edu

³Department of Environmental and Molecular Toxicology

Email: D_Shea@ncsu.edu

North Carolina State University

Raleigh, NC 27695 USA

Abstract: This presentation reviews four of the university analytical service centers currently located in the College of Agriculture and Life Sciences at North Carolina State University. Each service center houses a range of state-of-the-art analytical instrumentation that is available for support of research, teaching and extension faculty, students and staff. The policies regarding access to instrumentation and services varies among the three service centers, but all must charge for the services provided at rates approved bi-annually through the Office of Contracts and Grants at North Carolina State University. The supervisors of the respective service centers are tenured research faculty with substantial experience in the analysis of various sample matrices and in the development of suitable analytical protocols to successfully characterize unknowns. Technical support staff that are assigned to each service center carry out requested analyses and maintain the analytical instrumentation. In several centers, technical staff are available to train graduate students in the use of instrumentation so that they can carry out their own analyses for a modest usage fee.

The Environmental Analysis Laboratory is located in the Department of Biological and Agricultural Engineering (Weaver Labs, North Campus) and operates under the supervision of Dr. John Classen (515-6800). Analytical capabilities of the laboratory include macro- and micro-nutrients, oxygen demand, carbon content, and analyses of various gases that are important to waste management and environmental processes. All analyses are conducted by laboratory staff, who are committed to accurate and reliable analyses as demonstrated by rigorous quality control protocols.

The Analytical Service Laboratory (<http://www.soil.ncsu.edu/services/asl/>) is located in the Department of Soil Science (Williams Hall, North Campus) and operates under the supervision of Dr. Wayne P. Robarge (515-1454). The laboratory houses a range of analytical instrumentation including an ICP-optical emission spectrometer, flame atomic absorption spectrometers, continuous flow auto-analyzers, elemental analyzers, and ion chromatographs. The laboratory specializes in the elemental analyses of solids (especially plant and soil), and in the routine chemical analyses of water and wastewater. Several instruments (flame atomic absorption spectrometers and continuous flow auto-analyzers) are available for common use by staff and graduate students for a modest usage fee.

Also housed in Williams Hall is the Stable Isotope Mass Spectrometry Facility that utilizes the latest advances in Continuous Flow Isotope Ratio Mass Spectrometry to aid investigators in their research (<http://www.soil.ncsu.edu/services/sims/>). A variety of sample types can be analyzed and in one run data can be obtained for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, total %N and total %C. Both natural abundance and isotopically enriched samples can be measured on the system. Williams Hall will be renovated starting in July 2005. Both the Analytical Service Laboratory and the Stable Isotope Mass Spectrometry Facility will be moved to interim locations and remain operational during the renovation of Williams Hall. Please consult the respective WEB pages for information on how to access the centers from July 2005 to December 2006.

The Analytical Toxicology Laboratory is located in the Department of Environmental and Molecular Toxicology (850 Main Campus Drive, South Campus) and operates under the supervision of Dr. Damian Shea (513-3899). The laboratory provides analytical services in trace organic chemical analysis in environmental and biological samples. Instrumentation includes liquid chromatographs (LC), gas chromatographs (GC), and mass spectrometers (MS). The majority of analyses are conducted by LC/MS and GC/MS. These analyses include polycyclic aromatic hydrocarbons (PAHs) by GC/MS, polychlorinated biphenyls (PCBs) by GC and GC/MS, chlorinated and current use pesticides by GC/MS and LC/MS, antibiotics and other pharmaceutically active chemicals by GC/MS and LC/MS, phycotoxins by LC/MS, and many other synthetic chemicals. The laboratory also performs analysis on the identification and quantification of various metabolites of these exogenous chemicals. In addition, a new Small Molecule Laboratory is likely to emerge soon that consolidates other analytical services, including pesticide residue analysis, mycotoxin analysis, and sugar and carbohydrate analysis.

The College of Agriculture and Life Sciences at NC State is also developing a core analytical facility in metabolomics that will provide a broad scan of endogenous metabolites to allow identification of biomarkers of stress and to assist mechanistic research in whole organism response to stress (toxicant exposure, dietary deficiencies, heat, etc.). This laboratory will utilize primarily LC/MS techniques and will be coordinated with ongoing work in proteomics, genomics, and bioinformatics. The laboratory will be housed in the Department of Environmental and Molecular Toxicology and will be functional in winter/spring of 2005.

CONUNDRUM IV

Evaluating the nutritional status of animals is difficult at best, but in free living species it can be a very real problem. This conundrum is to discuss ways to assess nutritional status without the advantage of invasive sampling (such as blood samples). Consider two situations, one where the animal can be held in hand, and the other where the animal cannot be captured. How would you go about assessing nutritional condition?

NORTH CAROLINA STATE UNIVERSITY INTERDEPARTMENTAL NUTRITION GRADUATE PROGRAM

Jonathan C. Allen, Coordinator Nutrition Program

North Carolina State University, Box 7624, Raleigh, NC 27695-7624

Phone: (919) 515-2968, jon_allen@ncsu.edu

North Carolina State University's interdepartmental nutrition graduate program has six participating departments. These departments include: animal science, family and consumer science, food science, crop science, poultry science and toxicology. For a complete list of available faculty advisors within each department please visit the programs web page at: http://www.cals.ncsu.edu/food_science/acdprg/

The Nutrition Program is founded not only on advanced study in nutrition but also in related biological and physical sciences. Particular emphasis is given to the development of creativity in nutrition research. An individual program of courses, which includes certain core requirements, is developed for each student by an advisory committee.

Research activities are as diverse as the Nutrition faculty and range in level from the molecular to the whole animal. Students majoring in Nutrition are affiliated with and housed in one of the departments mentioned above. The choice of department, as well as faculty adviser, depends on the research interests of the student. Students have graduated from this program while working with both traditional livestock models as well as exotic species (such as numerous species of lemur).

Degrees Offered / Programs of study: Currently three difference degrees are available: A Master of Nutrition (Non-thesis), a Master of Science in Nutrition, and a Ph.D. in Nutrition. The programs of study generally fall within two categories, nutritional biochemistry and experimental animal nutrition. The requirements for each degree category as well as admission requirements are listed on the web page previously listed.

Available Graduate Courses in Nutrition: Nutrition Research Methods, Protein and Amino Acid Metabolism, Mineral Metabolism, Vitamin Metabolism, Energy Metabolism, Human Nutrition, Advanced Ruminant Nutrition, Advanced Special Problems in Nutrition, Nutrition and Biotechnology, Lactation, Milk, and Nutrition, Digestion and Metabolism in Ruminants, Food Lipids, Exercise Nutrition, and Feed Formulation and Simulation.

Every nutrition student is expected to have a strong background in chemistry and mathematics. Additional graduate courses in Biochemistry, Chemistry, Genetics, Microbiology, and Physiology are available for students to enhance the basic science support areas. A minor in another program or department is encouraged. Course selection for a plan of work is by agreement between the student and their faculty advisory committee.

(All information is taken from the North Carolina State University Interdepartmental Nutrition Graduate Program web page at: http://www.cals.ncsu.edu/food_science/acdprg/)

**SEASONAL VARIATION IN CLINICAL PATHOLOGY PARAMETERS AS
INDICATORS OF NUTRITIONAL STATUS OF IN-WATER JUVENILE
LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) IN NORTH CAROLINA**

Terra R. Kelly, DVM^{1,2,3,4*}, Joanne Braun-McNeill, MS⁵, Larisa Avens, PhD⁵, Matthew H. Godfrey, PhD⁶, Aleta A Hohn, PhD⁵, Ellis Greiner, PhD⁷, Craig A. Harms, DVM, PhD, DACZM⁸

1. Environmental Medicine Consortium, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606
2. Department of Clinical Sciences, North Carolina State University, 4700 Hillsborough Street, Raleigh, NC 27606
3. North Carolina Zoological Park, 4401 Zoo Parkway, Asheboro, NC 27205
4. corresponding author: terra_kelly@ncsu.edu, fax number (252) 222-6311
5. National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Beaufort Laboratory, 101 Pivers Island Road, Beaufort, NC 28516
6. North Carolina Wildlife Resources Commission, 1724 Mail Service Center, Raleigh, NC 27699
7. Department of Pathobiology, Building 1017, Room V3-240, 2015 SW 16 Ave., University of Florida, Gainesville, FL 32608
8. Center for Marine Sciences and Technology, 303 College Circle, Morehead City, NC 28557

In an ongoing sea turtle mark-recapture study, the health status of juvenile loggerhead sea turtles passively captured in stationary fishery pound nets in Core and Pamlico Sounds, North Carolina is being investigated using hematologic, biochemical, parasitologic, and toxicologic parameters. This project will provide a current index of population health and examine seasonal and multiple year trends.

Trends in the clinical pathology data are similar to that seen in juvenile loggerheads sampled within the same area during the fall of 1997 and summer of 2000. There is seasonal variation in some of the clinical pathology parameters. This seasonal variation is thought to be due to a relative fasting state of loggerhead sea turtles that are sampled during migration through the sounds of North Carolina in the fall.

METABONOMIC ASSESSMENT OF FATTY ACID PROFILES TO EVALUATE CORAL HEALTH

Michael K. Stoskopf, DVM, PhD, Dipl. ACZM.

Environmental Medicine Consortium, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St. Raleigh, NC 27606.

We are examining the suitability of biochemical profiling of total lipids and fatty acid methyl esters derived from neutral and phospholipids to assess the catabolic and metabolic health of coral colonies and evaluate the potential of corals to withstand environmental stressors and resist disease.

Lipids serve both structural and energy storage functions and are a main source of energy in coelenterata. Researchers have been investigating the dynamics of total lipid (TL) in corals over the past two decades (Harland et al 92). The total amount of lipids in storage reserve appears to indicate metabolic reserve, and may be a marker of the expected growth rate of corals. Reproductive effort and the TL content of the coral are interconnected. *Pocillopora damicornis* that brood larvae and produce ova increase their TL reserves prior to larval production and have lower growth rates than colonies that just produce sperm (Ward 1995). Coral planulae and gametes have very high TL content (50 to 70% lipid by mass)(Ward 1995). Increases in summer and autumn TL content appear to correlate with the number of embryos and higher fecundity observed at that time of the year in the soft coral *Heteroxenia fuscescens* (Ben-David-Zaslow and Benayahu 1999). Conversely, stresses that produce decreased growth of corals can result in increased reproduction. This in turn depletes TL stores of the colony. Abiotic water factors also affect TL content of corals. Summer stratified warm water can be poor in nutrients, while winter upwelling low temp water rich in nutrients can help build lipid energy reserves for some species (Ben-David-Zaslow and Benayahu 1999). Depth and light availability can also affect TL content of corals. In shallower water, *Porites porites* routinely has about 11% of dry weight as lipid, while the same species in deeper, darker water has about 8.5 % TL (Harland et al. 1992). Therefore, TL may serve as a valuable indicator of coral energetic reserves with the expectations that corals with lower TL content would be more susceptible to emerging coral diseases.

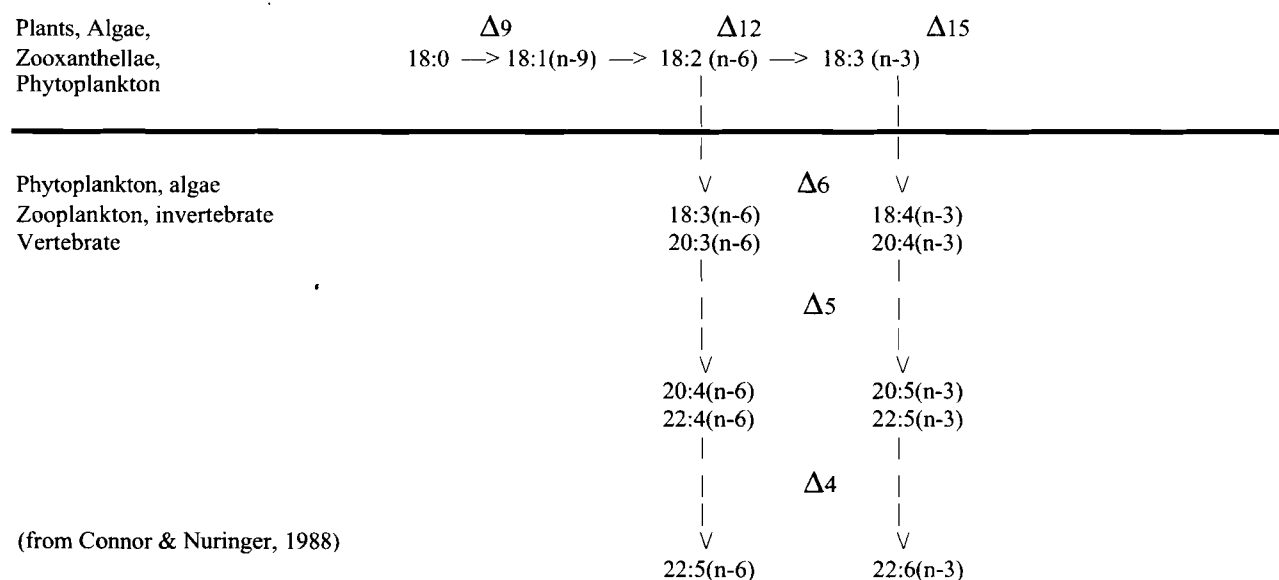
To dissect the basis for fluctuations in total lipid content and refine the interpretation of the lipid profile's relationship to metabolic health of coral more sophisticated evaluations of the lipids of coral are needed. Examination of fatty acid profiles can provide important insights into the origin of lipid stores and the relative activity of various enzymatic steps of the key metabolic pathways of corals (figure 1). The major fatty acids of corals, across the many species studied are consistently 16:0, 18:0, 18:1(n-9), 20:4(n-6), 20:5(n-3), and 22:6(n-3) (Latyshev, 1991) and specific fatty acids appear to be characteristic of coral families, though use of the technique for taxonomics has not been fruitful. This has been primarily because of the variations infused by alterations in metabolic balances (Harland et al 1992). These variations, that pose problems for taxonomists, are precisely the perturbations that make lipid and particularly FAME profiles of corals potentially useful for health assessment.

The fatty acid profile of a coral will be affected by the prevalent food source (Harland et al 92). Zooxanthellae to host transfer of glycerol and lipids, including polyunsaturated fatty acids

(PUFA) occurs (Patton et al 1977). PUFA is commonly considered indicative of external food sources, though it is recognized that PUFA from photosynthetic activity of zooxanthellae and from biosynthesis from coral also contribute to the PUFA pool in stored lipid reserves in coral. It is possible, however, to tease out the contribution of various sources of lipid through careful examination of the ratios of different PUFA in the fatty acid profiles of a coral. Likewise, lipids in zooxanthella can be evaluated. This capability is the result of differential processing in the fatty acid metabolic pathways between coral and zooxanthellae (figure 1) and allows the fatty acid profile of a coral to serve as an indicator of the metabolic status of both the photosynthetic symbiont and the coral itself. For example, depth is thought to affect the degree of n-3 and n-6 of unsaturation in neutral lipids contributed by zooxanthellae because of differences in light availability at different depths. The majority of neutral lipids in coral are stored in the form of lipid droplets which contain an unusually high percentage of saturated fatty acids for marine organisms. The zooxanthellae driven changes in n-3/n-6 saturation patterns are not seen, however, in the phospholipid fraction of coral total lipids (Latyshev 91) which are primarily found in the structural membranes of the coral. Polyunsaturated fatty acids (PUFA) are particularly promising markers because both n-6 and n-3 PUFA are necessary for healthy function and normal activity coral. The scleractinian coral in the family Acroporidae, are characterized by large amounts of n-6 polyunsaturated fatty acids (PUFA) rather than the predominance of n-3 PUFA typical in marine species. The characteristic PUFA's of the acroporidae are thought to be 18:3(n-6), 18:4(n-3) and 22:4(n-6)(Latyshev, 1991). It is reasonable to hypothesize that a deficit of $\Delta 5$ -desaturase activity would cause an increase in the pool of 20:3 (n-6) and 20:4(n-3) but a decrease in 20:4(n-6) and 20:5(n-3), suggesting that this pattern in acroporid corals is a result of the metabolic pattern of the coral itself and not its zooxanthellae (see figure 1). Carefully analyzed FAME profiles with specific attention to differentiating PUFA content may provide a practical means to evaluate the metabolic vigor of a coral colony and provide insight into coral susceptibility to disease.

Figure 1.

Fatty Acid Metabolic Pathways of Desaturation



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CONUNDRUM V

Local bear hunters have taken up the practice of putting large blocks of waste candy out to attract bears to their hunting areas. The blocks of candy weigh over 1 ton and are placed out year round. The bears are attracted to the candy and some bears eat it continually. If you were asked how to evaluate the health impacts of such a practice on the bears by an agency interested in eliminating the practice, how would you study the problem?

SURVEY OF GIRAFFE DIET AND HEALTH HISTORIES AMONG NORTH AMERICAN ZOOS

Kathleen Sullivan,¹ Eric van Heugten,¹ PhD, Kimberly Ange-van Heugten,¹ MS, Barbara Wolfe,^{2,3} DVM, PhD, DACZM

¹Department of Animal Science, North Carolina State University, Raleigh, NC 27695

²North Carolina Zoological Park, 4401 Zoo Parkway, Asheboro, NC 27205

³College of Veterinary Medicine, North Carolina State University

Obstructive urolithiasis is a significant health risk in adult giraffe in captive collections. Urolithiasis is the development of urinary calculi. These stones, which can be composed of a combination of phosphorus, calcium and magnesium, may obstruct the urethra and lead to bladder rupture and death. The condition has been well documented in domestic ruminants such as goats,¹ as well as in giraffe.² Based on previous investigations, key contributing factors to urolithiasis in giraffe may be improper dietary mineral balance; increased urine pH, and low water intake leading to concentrated urine.² In order to further investigate the possible causes of this problem, a comprehensive survey was initiated in September 2004 of North American giraffe collections within zoological institutions, which looked specifically at giraffe feeding practices and medical histories. The survey also aimed to ask institutions for aid in obtaining samples of feeds, water, serum, urine and feces, as well as inquiring into their possible participation in a feeding trial.

Currently, 25 institutions have sent in preliminary responses of the 95 institutions contacted through the giraffe American Zoo and Aquarium Association Species Survival Plan program. A total of 107 giraffe were accounted for with 34 males and 73 females. From the survey, 39 of the total giraffes were known to be reticulated (*Giraffa camelopardalis reticulata*), 4 were known to be subspecies hybrids, and 15 were known to be masai (*Giraffa camelopardalis tippelskirchi*). Of the males 38% were under 4 years, 44% were between 4 and 20 years of age, 9% were over 20 years, and 9% were of unknown age. The females had 16% under 4 years of age, 40% between 4 and 20 years of age, 21% over 20 years, and 23% of unknown age. Under four years of age, giraffes are not fully sexually mature and over 20 years of age, females are generally considered to no longer able to reproduce.³ Twelve percent of the institutions housed their giraffes with other species making nutritional investigations more difficult. Seventy-two percent of the 25 institutions feed their giraffes in groups. However of the zoos that group-feed, 39% (7/18) fed the one male in their collection separately from the herd and 27% (5/18) group-feed during the day but allow single feeding at night.

Regarding the diets of the giraffes, all institutions responded that they fed concentrate and browse at least seasonally. Approximately 40% of the institutions feed the same concentrate (Mazuri ADF 16 "low fiber" pellets, PMI Nutrition International, St. Louis, MO 63166), but the rest vary greatly in pellet used. Almost all (24/25 or 96%) of the institutions fed hay, 84% (20/24) of which fed alfalfa hay from varying regions. All institutions fed some form of supplement and (or) a form of treat. Seventy six percent (19/25) fed a mineral block to the giraffes, and 16% (4/25) fed a salt block only. The remaining 8% used other supplementation

such as vitamin E. When asked about water sources, 72% of the zoos were on city water, and 28% had ground wells.

In terms of the health histories provided, 5 of the institutions noted a problem with urolithiasis, 7 noted issues with wasting and 8 noted possible peracute mortality. The 25 institutions noted 10 other occurrences of various medical symptoms, such as blood on the prepuce and renal disease. Only 4 of the 25 institutions responded that they had never encountered medical problems. The giraffes found with urolithiasis reportedly had stones consisting of calcium phosphate (carbonate form), magnesium-calcium phosphate and calcium oxalate.

Twenty institutions have offered to consider helping in a feeding trial when more information about what it would involve was given. Twelve of the 25 institutions can help us by providing samples of feeds, water, serum, urine or feces. Five institutions would be unable to aid our investigation due to various reasons, including an inability to separate animals for a trial and sick animals.

Additional completed surveys are being collected and added to the database. Further, we are currently formulating plans for the experimental phase of this project. Samples of feeds, water, serum, urine and feces will be collected and analyzed for all institutions that are willing to help. Follow up surveys will be sent out as well, to obtain more specific information on the amounts and types of feed provided. Along with the continually incoming surveys, and the samples, a preliminary feeding trial will occur at the Asheboro Zoo. The comprehensive plan aims to gain insight into the nutritional factors that may incite urolithiasis in giraffes and correct them.

Acknowledgements: The authors would like to thank Mazuri (PMI Nutrition International) and the American Association of Zoo Veterinarians for their financial assistance with this continuing project.

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DIABETES PARAMETERS OF WOOLLY MONKEYS (*LAGOTHRIX LAGOTRICHA*) AS INDICATORS OF HEALTH STATUS

Kimberly Ange-van Heugten, M.S.*¹

Walter Jansen, Ph.D.²

Martin Verstegen, Ph.D.³

Roy Burns, D.V.M.⁴

Eric van Heugten, Ph.D.¹

¹*North Carolina State University, Department of Animal Science, Raleigh, NC 27695-7621, USA*

²*European Zoo Nutrition Centre, c/o EAZA Executive Office, P.O. Box 20164 NL-1000 HD
Amsterdam, The Netherlands*

³*Wageningen University, Building 531, Zodiac, Marijkeweg 40, 6709 PG Wageningen, The
Netherlands*

⁴*Louisville Zoo, P.O. Box 37250, Louisville, KY, USA 40233-7250*

Introduction

The woolly monkey is a threatened species (Nowak, 1999). Their threatened status is especially problematic because they are increasingly sought after as both a source of food for their native people and as a popular monetary income by capturing and selling them to the pet trade. In addition, these animals reproduce slowly compared to many primate species and they are not tolerant of habitat destruction.

To conserve the species, numerous zoos have attempted to house successful breeding populations. Many of these zoos have not been successful for various reasons. The most common causes of death in captive woolly monkeys are pregnancy complications and hypertension related conditions such as congestive heart failure, renal failure, and cardiovascular events (Giddens, 1987; Miller et al., 1995).

It has been reported that woolly monkeys also suffer from diabetes mellitus (Type II) or unique carbohydrate metabolism issues that currently detrimentally affect their captive health (Vermeer, 1994). This condition, very similar to humans, is thought to worsen with pregnancy. Literature regarding diabetes in woolly monkeys is very sparse (although it is a widely believed concern in practice). The current experiment was conducted to evaluate diabetic parameters of a woolly monkey population to ascertain whether diabetes is a genuine concern or if research should proceed into different areas to ensure successful captive management of this unique animal.

Data Collection

Three-day diet disappearance information and blood and urine samples were collected from six woolly monkeys housed at The Louisville Zoo, Kentucky, USA. All animals were housed in their usual exhibit areas and were fed diets that were considered nutritionally adequate and normal. Animals were fasted overnight prior to immobilization, and veterinary staff collected blood into tubes appropriate for each chemical analysis. The protocol was performed according to the Louisville Zoo's animal care guidelines. The blood was analyzed for fructosamine and insulin (Antech Diagnostics, Alsip, Illinois, 60803 USA), glycated hemoglobin (Louisiana Veterinary Medical Diagnostic Laboratory, Baton Rouge, Louisiana, USA), and lipid profile (total cholesterol, triacylglycerides, HDL-cholesterol and LDL-cholesterol); (The Simian Diagnostic Laboratory, Rockville, MD, USA.).

Results and Discussion

This study did not show any clear nutrient deficiencies in the daily diets. With the exception of HDL-cholesterol, all blood measurements were within reported normal ranges for humans and similar non-human primate species (Table 1). In addition, the urine from all of the woolly monkeys within this study tested negative for glucose although the daily diet was not limiting in available sugars. From these measurements, it does not appear that diabetes mellitus is a problem within this sample of woolly monkeys and therefore diabetes is likely not a primary link to the health problems facing the woolly monkey.

It is important to note that our study only had one female and she was not pregnant and therefore we cannot make any gestational diabetes observations. In addition, all of the monkeys in this research trial are taking daily medication for high blood pressure that could potentially alter blood measurements. HDL-cholesterol appeared low for five of the six woolly monkeys within this study and this is likely a result of both the diet and the medications previously discussed.

Future research is needed to ascertain the true nature of the problems faced by this species. We plan to continue studying the carbohydrate metabolism link in woolly monkeys receiving diets with known composition and diet intake in an effort to ascertain the effects that potential stressors may have upon health status in this primate.

Acknowledgments

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Table 1. Serum measurements of woolly monkeys at The Louisville Zoo for diabetic parameters and published reference values for various other primate species.

Woolly Monkey	Sex	Age yr	Fructosamine $\mu\text{mol/L}$	Glucose mmol/L	Glycated Hb %	Insulin $\mu\text{U/mL}$	Triglycerides mg/dl	Cholesterol mmol/L	HDL-Chol mmol/L	LDL-Chol mmol/L
1	Male	8	220	4.72	3.52	13.0	0.46	2.5	0.4	1.8
2	Male	15	219	2.22	4.73	6.2	0.58	3.3	0.4	2.7
3	Male	11	193	3.83	3.78	8.6	0.42	5.1	1.6	0.4
4	Female	3	203	4.78	4.05	7.0	0.38	3.0	0.8	2.2
5	Male	14	139	3.50	4.49	8.0	0.71	3.3	0.4	2.6
6	Male	16	242	4.39	3.61	6.6	0.60	4.7	1.0	3.4
Average			203	3.91	4.03	8.2	0.52	3.6	0.8	2.2
Published Reference Values										
Spider Monkey & related species ^{1,2}			NR	NR	NR	NR	0 - 1.7	0 - 5.6	≥ 1.2	0 - 4.0
New World Primates ³			NR	NR	4.9 \pm 0.65	NR	NR	NR	NR	NR
Human ⁴			0-285 ^{5,6}	< 6.11 ⁷	NR	5 - 20	< 1.7	< 5.1	≥ 1.0	< 2.6

¹ Crissey et al., 1999

² The Simian Diagnostics Labs reported new world primate reference values

³ Dutton et al., 2003

⁴ Health Net Inc., 2004

⁵ Merck Selectra, 1996

⁶ Guthrie and Guthrie, 2004

⁷ < 6.11 normal, 6.11 - 7.00 glucose intolerant, >7.00 diabetic

MILK COMPOSITION IN RING-TAILED AND RED-FRONTED LEMURS

C. Tilden¹, PhD, M. L. Power^{2*}, PhD, M. E. Pereira³, PhD, and O. T. Oftedal², PhD

¹Department of Anthropology, University of Kansas, Lawrence, KS, 66045-7556, USA,

²Smithsonian's National Zoological Park, ³The Latin School of Chicago

Lactation is a defining characteristic of mammals, and a lactation strategy is an important component of a mammalian species' life history strategy, affecting both maternal cost of reproduction and infant growth. Milk composition is one aspect of a lactation strategy, along with milk production, nursing frequency, and length of lactation. These aspects together serve to deliver the necessary nutrition to support infant growth, while remaining within the constraints of maternal metabolic capabilities.

Until recently little was known about the energetic expense of lactation in lemurs, despite that aspects of life history, physiology, and behavior are believed to have evolved due to selection pressures pertaining to high female reproductive costs. Reproductive seasonality, birth synchrony, and rates of pre- and post-natal growth have been suggested to relate to costs of lemur lactation. Several true lemurs (*Eulemur* spp.) minimize the daily costs of lactation by producing dilute milks in small volumes. Previous data suggested that the milk of ring-tailed lemurs (*Lemur catta*) might be higher in fat, protein and energy than that of *Eulemur* spp., despite the many similarities between these taxa. This study sought to compare the composition of milk of *Lemur catta* to that of red-fronted lemurs (*Eulemur fulvus rufus*), and to examine milk composition over lactation in both species.

Methods

Milk samples were obtained from seven individual semi-free-ranging ring-tailed (*Lemur catta*) and two individual red-fronted lemurs (*Eulemur fulvus rufus*) at approximately two, four and five months postpartum. Milk samples were assayed for dry matter (DM), nitrogen, fat, and sugar at the Nutrition Laboratory of the Smithsonian's National Zoological Park using standard methods. Crude protein (CP) was calculated by $6.38 \times$ nitrogen, and gross energy (GE) was calculated using 5.86 kcal/g CP , 9.11 kcal/g fat , and $3.95 \text{ kcal/g sugar}$.

Results for each nutrient are given as mean \pm SE. The variation in DM, CP, fat, sugar, and GE concentrations of *Eulemur fulvus rufus* and *Lemur catta* milk was examined by multivariate analysis of covariance, with species as the categorical variable, and days postpartum as the covariate. Differences in DM, CP, fat, sugar, and GE concentrations of milk between mid and late lactation (after 150 days postpartum) were examined using one-way multivariate analysis of variance.

Results

For mid-lactation milk (day 39 to day 137 postpartum) there were no significant differences between *L. catta* (DM=10.7 \pm 0.2%, CP+1.6 \pm 0.1%, fat=0.9 \pm 0.1%, sugar=7.9 \pm 0.1%, GE=0.49 \pm 0.02) and *E. fulvus rufus* (DM=10.5 \pm 0.1%, CP+1.5 \pm 0.1%, fat=0.5 \pm 0.1%, sugar=7.8 \pm 0.1%, GE=0.44 \pm 0.01), although milk fat (p=.064) and gross energy (p=.050) tended to

be higher in *L. catta*. In both species late-lactation milk (beginning 150 days postpartum) was significantly higher in dry matter ($p=.022$) and crude protein ($p=.007$) and lower in sugar ($p=.013$) than mid-lactation milk.

Discussion

Milk composition did not differ between ring-tailed lemurs and red-fronted lemurs. Milk low in DM, fat, CP and GE appears to be characteristic of most, if not all, *Eulemur* and *Lemur* spp., and represents one of a suite of traits that minimize daily reproductive costs.

Milk composition varied between mid and late lactation, with late lactation milk being higher in fat and protein. Adaptive explanations are tempting. For example, we could hypothesize that older, and hence larger and less vulnerable, infants both require more nutrition and are evolutionarily “worth” more to females. However, infants of this age are self-feeding and nurse less frequently. Perhaps the change in milk composition is due to milk solids accumulating in the mammary gland because of incomplete or less frequent evacuation. Without a measure of milk intake by infants at late lactation, the change in milk composition cannot be said to result in a greater nutrient transfer from mother to infant.

Attendee List

*Anne Acton, DVM
NC State University
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, NC 27606-1499
aacton@ncsu.edu

*Jonathan C. Allen
NC State University
Food Science
Box 7624
Raleigh, NC 27695-7624
jonathan_allen@ncsu.edu

*Kimberly Ange
NC State University
Animal Science
Box 7621
Raleigh, NC 27695-7621
kim_ange@ncsu.edu

Jim Atkinson, PhD
University of Guelph
Dept. of Animal & Poultry Science
Guelph, Ontario
N1G 2W1 Canada
jatkinso@uoguelph.ca

Rick Bertram, DVM
GlaxoSmithKline
Five Moore Drive
Research Triangle Park, NC 27707
rick.h.bertram@gsk.com

Julie Cavin
NC State University
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, NC 27606-1499
jmcavin@unity.ncsu.edu

Charles Coats, DVM
4710 Sunset Avenue
Rocky Mount, NC 27804
coatsvet@coatsvet.com

*Adam Craig
University of Guelph
Dept. of Animal & Poultry Science
Guelph, Ontario
N1G 2W1 Canada

Sally Davis
NC State University
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, NC 27606-1499
asdavis2@ncsu.edu

Cheryl L. Engfehr
Michigan State University
635 Abbott Road, Apt. 320
East Lansing, MI 48823
engfehrc@msu.edu

*Laura Felicetti, PhD
Zoological Society of San Diego
PO Box 120551
San Diego, CA 92112-0551
lfelicetti@sandiegozoo.org

Peggy Ferebee
Natural Science Center of
Greensboro
4301 Lawndale Drive
Greensboro, NC 27455
pferebee@natsci.org

Esther Finegan, PhD
University of Guelph
Dept. of Animal & Poultry Science
Guelph, Ontario
N1G 2W1 Canada
efinegan@uoguelph.ca

Melinda Frankus
The Phoenix Zoo
455 North Galvin Parkway
Phoenix, AZ 85005
mfrankus@thephxzoo.com

Harold Furr, PhD
Craft Technologies
4344 Frank Price Church Road
Wilson, NC 27893
hfurr@crafttechnologies.com

Sarah Gabris
NC State University
310D Bragaw Hall
NCSU Box 15329
Raleigh, NC 27607
shgabris@unity.ncsu.edu

*Michele Gaffney
Zoological Society of San Diego
PO Box 120551
San Diego, CA 92112-0551
migaffney@aol.com

Tyler Greene
NC State University
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, NC 27606-1499
tbgreene@ncsu.edu

Craig A. Harms, DVM, PhD
NC State University
CMAST
303 College Circle
Morehead City, NC 28557
craig_harms@ncsu.edu

Lindsey M. Hendricks
The Phoenix Zoo
5937 East Sheena Drive
Scottsdale, AZ 85254
tywebb281@hotmail.com

*M. Elizabeth Hile
North Carolina Zoo
4401 Zoo Parkway
Asheboro, NC 27205
liz.hile@ncmail.net

Harold Hintz, PhD
Cornell University
345 Morrison Hall
Ithaca, NY 14853-4801
hfhl@cornell.edu

Jonathan Holt
NC State University
Raleigh, NC 27695
jpholt@ncsu.edu

Wendy Hood, PhD
Coastal Carolina University
938 Marsh Field Circle, #204
Myrtle Beach, SC 29579
wrhood@coastal.edu

Bob Hyde
Nashville Zoo
3777 Nolensville Road
Nashville, TN 37211
rhyde@nashvillezoo.org

*Terra Kelly, DVM
NC State University
College of Veterinary Medicine
4700 Hillsborough Street
terra_kelly@ncsu.edu

Suzanne Kennedy-Stoskopf,
DVM, PhD
NC State University
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, NC 27606-1499
suzanne_stoskopf@ncsu.edu

Melanie Landry
NC State University
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, NC 27606-1499
mmlandry@ncsu.edu

Nancy Langwith, DVM
1508 East 86th Street PMB 320
Indianapolis, IN 46240
kirschnj@aol.com

Gregory A. Lewbart, VMD
NC State University
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, NC 27606-1499
greg_lewbart@ncsu.edu

*Barbara Lintzenich, MS
Brookfield Zoo
3300 Golf Road
Brookfield, IL 60513
balintze@brookfieldzoo.org

Shannon Livingston, MSc
Toronto Zoo
361 A Old Finch Avenue
Scarborough, Ontario
M1B 5K7 Canada
slivingston@torontozoo.ca

Debbie Lowe
Zoological Society of San Diego
PO Box 120551
San Diego, CA 92112-0551
dlowe@sandiegozoo.org

Gary Lynch, PhD
Land O'Lakes/Mazuri
555 Maryville University Drive
St. Louis, MO 63141
glynch@landolakes.com

*Robert MacLean, DVM
NC State University
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, NC 27606-1499
drmaclean2000@yahoo.com

Ellen J. Magid, DVM
USDA, APHIS, AC
920 Main Campus Drive
Raleigh, NC 27606
ellen.j.magid@aphis.usda.gov

Michael L. Mahalak
North Carolina Zoo
4401 Zoo Parkway
Asheboro, NC 27203
michael.mahalak@ncmail.net

Jeannette Moore, PhD
NC State University
Animal Science
Box 7621
Raleigh, NC 27695-7621
jeannette_moore@ncsu.edu

*E. Wilson Myers
Colorado College
902 N Cascade WB 521
Colorado Springs, CO 80946
ewilsonm@yahoo.com

*Michael L. Power, PhD
Smithsonian National Zoo
Washington, DC 20008
powerm@nzp.si.edu

Debbie Rea
Maymont Foundation
1700 Hampton Street
Richmond, VA 23220
drea@maymont.org

Christine Ann Remington, MS
Alamance Community College
1247 Jimmie Kerr Road
Graham, NC 27253-8000
remingc@alamance.cc.nc.us

*Alejandra Renjifo
Disney's Animal Kingdom
PO Box 10,000
Lake Buena Vista, FL 32830
alejandra.renjifo@disney.com

*Wayne Robarge, PhD
NC State University
Soil Science
Box 7619
Raleigh, NC 27695-7619
wayne_robarge@ncsu.edu

Christina Rush
NC State University
310D Bragaw Hall
Box 15329
Raleigh, NC 27607
cmrush@unity.ncsu.edu

Heather Sallade, DVM
6240 St. Regis Circle, Apt. 202
Raleigh, NC 27606

*Michael L. Schlegel, PhD
University of Florida
6641 Banner Lake Circle Apt. 9201
Orlando, FL 32821-7359
schlegel@animal.ufl.edu

Deb Schmidt, PhD
Lincoln Park Zoo
2001 North Clark Street
Chicago, IL 60614
dschmidt@lpzoo.org

Michelle Shaw, MSc
Toronto Zoo
361 A Old Finch Avenue
Scarborough, Ontario
M1B 5K7 Canada
mshaw@torontozoo.ca

Thomas Sheridan, DVM
South Carolina Aquarium
1038 Folly Road
Charleston, SC 29412
tsheridan@scaquarium.org

Anna-Kate Shoveller, PhD
University of Guelph
Dept. of Animal & Poultry Science
Guelph, Ontario
N1G 2W1 Canada

*Michael K. Stoskopf, DVM, PhD
NC State University
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, NC 27606-1499
michael_stoskopf@ncsu.edu

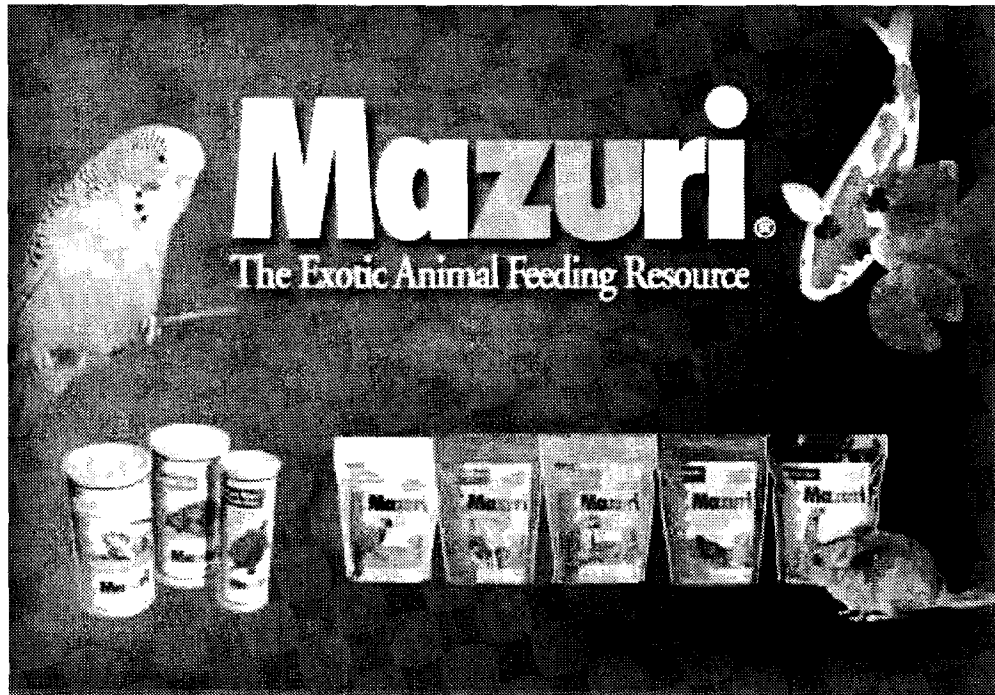
*Kathleen Sullivan
NC State University
5000 Ft. Sumter Road Apt. M
Raleigh, NC 27606
kesulli2@ncsu.edu

*Maryanne E. Tociłdowski, DVM
Houston Zoo
1513 North McGregor
Houston, TX 77030
mtocidłowski@houstonzoo.org

*Allison D. Tuttle, DVM
NC State University
College of Veterinary Medicine
4700 Hillsborough Street
Raleigh, NC 27606-1499
allison_tuttle@ncsu.edu

Eric van Heugten, PhD
NC State University
Animal Science
211-A Polk Hall
Box 7621
Raleigh, NC 27695-7621
eric_vanheugten@ncsu.edu

Rhonda Washington
UNCG School of Education
320 Severin Street
Chapel Hill, NC 27516
rlwashin@uncg.edu



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