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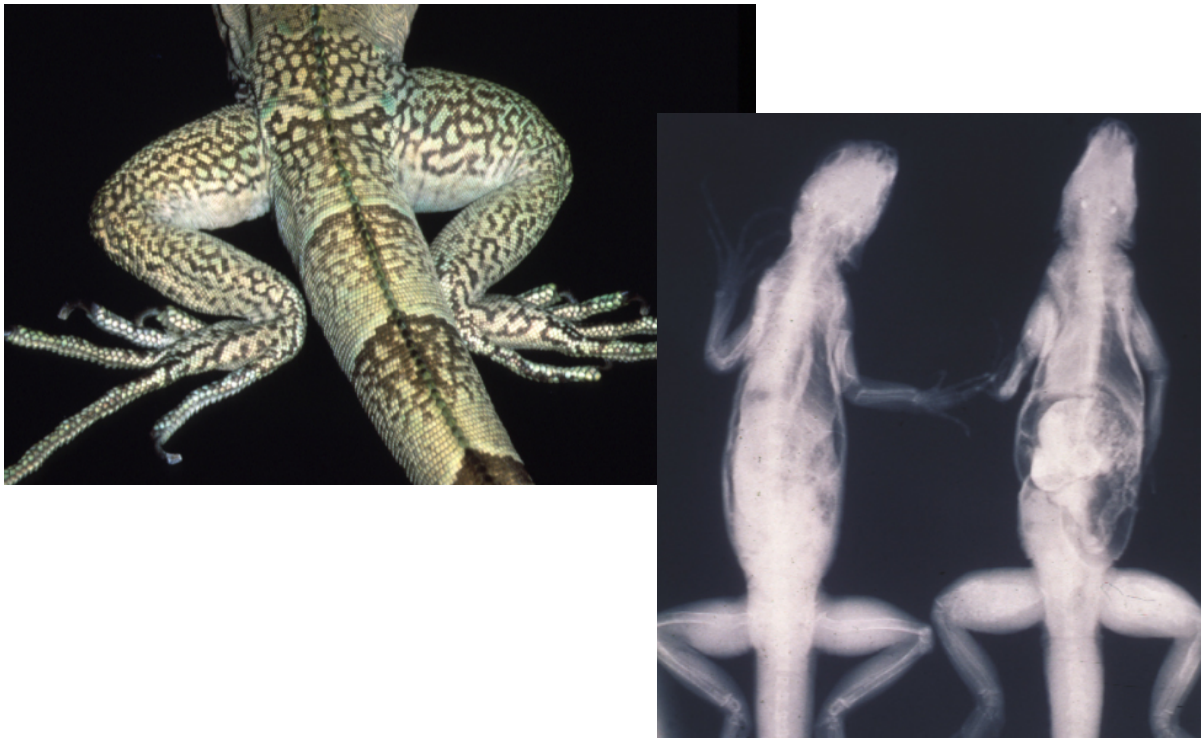

Crissey Zoological Nutrition Symposium

Raleigh, North Carolina

December 7 and 8, 2012

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Seventh Crissey Zoological Nutrition Symposium
Raleigh, North Carolina
December 7 and 8, 2012

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, bottlenosed 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 who's enthusiastic joy 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 her 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

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Friday
December 7, 2012

			Page
12:30 – 1:00	Registration		
1:00 – 1:30	Conference Introduction and Welcome	Michael Stoskopf Eric van Heugten	

Session I: MicroNutrient Nutrition & Nutrient Requirements
Session Chair: Dr. Peter Ferket

1:30 – 1:45	Dietary Management and Avian Rickets in the Pekin Robin, *Leiothrix Lutea*	Gjeltema & DeVoe	1
1:45 – 2:00	Changes in Plasma Calcium to Phosphorus Rations in Rehabilitating Green Sea Turtles in Response to Dietary Alterations	Christiansen et al.	4
2:00 – 2:15	Elemental Composition of Commercially Available Whole Prey: Comparison to Requirements of Dogs and Cats	Kerr & Swanson	7
2:15 – 2:30	Metanutrition: Using Metagenomics to Analyze the Gut Microbiome	McKenney et al.	9
2:30 – 2:45	Question and Answer Session		
2:45 – 2:50	Presentation of Conundrum I - Vitamin E Formulations and Supplementation Rates	van Heugten	11
2:50 – 3:20	**Break & Poster session**	**Blue Commons**	

Session II: Nutrient Requirements Continued
Session Chair: Dr. Paul Siciliano

3:20 – 3:35	Discussion of Conundrum I	van Heugten	
3:35 – 3:50	The Potential Effects of Diet and Environmental Stressors on Cortisol Response in the captive Southern Rhinoceros (*Ceratotherium Simum Simum*) at the North Carolina Asheboro Zoo	Ellis et al.	13
3:50 – 4:05	Application of Nuclear Magnetic Resonance Spectroscopic Metabolomics to Questions of Bivalve Nutrition	Hurley-Sanders et al.	16

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4:05 – 4:20	Nutrient Supplementation of Amnion Fluid by In Ovo Feeding Enhances Avian Perinatal Development and Post-Hatch Growth	Ferket	18
4:20 – 4:35	Digestible Energy Intake and Digestive Efficiency of Captive North American River Otters (*Lontra canadensis*)	Minter et al.	20
4:35 – 4:50	Question & Answer Session		
4:50 – 4:55	Presentation of Conundrum II - How is Metabolic Bone Disease Still a Problem?	McComb-Renjifo	23
4:55 – 6:00	**Evening Reception with Light Refreshments**	**Green Commons**	
6:00 – 7:00	Key Note Address		

Assessment of Vitamin and Mineral Status in Wild Animals
by
Dr. Eduardo Valdes

Saturday December 8, 2012			Page
8:00 – 9:00	**Continental Breakfast**		

Session III: General Nutrition
Session Chair: Dr. Jack Odle

9:00 – 9:15	Discussion of Conundrum II	McComb-Renjifo	
9:15 – 9:30	Hepatic Metabolomic Investigation of the North American Black Bear (*Ursus americanus*) Using 1H-NMR Spectroscopy	Niemuth & Stoskopf	25
9:30 – 9:45	Academic Perspectives on Helping Students Conduct Exotic Animal Research	Ange-van Heugten et al.	27
9:45 – 10:15	Manufacturing Feeds for Exotic Animals	Koutsos	30
10:15 – 10:30	Question and Answer Session		
10:30 – 10:50	**Break**		

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	Session IV: General Nutrition - Obesity Session Chair: Dr. Liz Koutsos		
10:50 – 11:05	Metabolic Consequences of the Early Onset of Obesity in Common Marmoset Monkeys	Power et al.	32
11:05 – 11:20	Ossabaw and Commercial Swine as Models for Juvenile Obesity Research	Seabolt	37
11:20 – 11:50	Transcutaneous Rump Ultrasound of Asian Elephants (Elephas maximus): Body Fat, Body Condition and Body Weight	Treiber et al.	40
11:50 – 12:05	Question and Answer Session		
12:05 – 12:10	Presentation of Conundrum III - Interpreting Metabolomics Data for Free-ranging Animals	Pratt - Phillips	47
12:10 – 1:35	**Lunch**	**Green Commons**	
	Session V: General Nutrition Session Chair: Dr. Korinn Saker		
1:35 – 1:50	Discussion of Conundrum III	Pratt - Phillips	
1:50 – 2:05	Development of Methods for Assessing Great Ape Body fatness	Reppert et al.	49
2:05 – 2:20	Hand- rearing Monk Parrots (*Myiopsitta monachus*)	Petzinger	55
2:20 – 2:35	Effects of processing time on blood and plasma samples from loggerhead sea turtles (*Caretta caretta*) for 1H-NMR-based metabolomics	Niemuth et al.	59
2:35 – 2:50	Effect of Reduced Animal-Based Protein and Total Protein in the Captive Diet of Maned Wolf	Kendrick et al.	62
2:50 – 3:05	Question and Answer Session		
3:05 – 3:25	**Break**		

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Session VI: General Nutrition

Session Chair: Dr. Vivek Fellner

POSTERS

DIETARY MANAGEMENT AND AVIAN RICKETS IN THE PEKIN ROBIN, *LEIOTHRIX LUTEA*

Jenessa Gjeltema, DVM[1,2,3] and Ryan De Voe DVM, Dipl. ACZM, ABVP – Avian[1,2,3]

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Avian rickets is a serious developmental disease that is associated with abnormal bone development in a variety of avian species. The disease is characterized by insufficient mineralization of bone during growth, which leads to the clinical manifestations of rickets such as lameness, abnormal flexibility of bones, and tibial dyschondroplasia. Dietary deficiencies in calcium, phosphorous, and vitamin D3 lead to the abnormal calcification of bone.

Histologic evaluation of the bones of affected animals can reveal thickened growth plates, disorganized mineralization, abnormal retention of cartilage, and changes to the epiphyseal vasculature. Hypocalcemic rickets can be identified histologically by an accumulation of proliferating chondrocytes with increased length of perforating epiphyseal vessels. In contrast, hypophosphatemic rickets is usually associated with an accumulation of hypertrophic chondrocytes.

As a part of its avian propagation program, the North Carolina Zoological Park manages the reproduction of a number of small passerine species including the Pekin Robin, *Leiothrix lutea*. Historically, this species has had poor nestling and fledgling survival, with a large proportion of histologically evaluated animals having lesions attributable to avian rickets. The North Carolina Zoological Park has instituted several management and dietary changes for this species since 2007 with the majority of changes occurring in 2010 and 2011.

During the breeding season, the diet had historically included Mazuri® Small Bird Breeder diet, Mazuri® ZuLiFe bird gel, a mixture of fruit and vegetables, and larvae of *Hemetia illucens* dusted with Avia vitamin supplement. One change made to the diet included replacing *Hemetia illucens* larvae with a mixture of waxworms, mealworms, superworms, and crickets. Additional changes also included feeding the provided crickets and mealworms a high calcium diet, replacement of Zeigleir® Bird of Paradise pellets with Marion ScenicTM Jungle & Paradise Bird Food, and dusting of insects with calcium carbonate rather than Avia brand vitamin supplement.

Taxonomic records for 117 hatched *Leiothrix lutea* were reviewed from 2006 through 2012 to determine annual survival rates past 3 months of age. Pathology reports including gross findings, histopathologic findings, final diagnoses, and cause of death were reviewed for all deceased birds during this time period. Animals with gross or histopathologic findings consistent with the disease were categorized as cases of avian rickets. Following the implementation of the dietary changes in 2010 and 2011, there have been fewer documented cases of avian rickets. This presentation discusses the dietary changes that were implemented for this species between 2010

and 2011 and how these changes may relate to the decreased numbers of documented cases of avian rickets in Pekin Robins at the North Carolina Zoological Park.

Acknowledgements: I sincerely appreciate the contributions of Kim Moses, Stephanie Krueger, Wendy Wadsworth, and Debbie Zombeck in providing details about the dietary and management changes that were implemented for this species. I would also like to acknowledge Judy Hunt for her assistance with obtaining the pathology records that were used in this study. I also appreciate Dr. Michael Stoskopf's input and suggestions during the preparation of this abstract.

CHANGES IN PLASMA CALCIUM TO PHOSPHORUS RATIOS IN REHABILITATING GREEN SEA TURTLES IN RESPONSE TO DIETARY ALTERATIONS

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The Karen Beasley Sea Turtle Rescue and Rehabilitation Center (KBSTRRC) in Topsail Beach, North Carolina admits between 15 and 45 native sea turtles each year. Many of these undergo prolonged rehabilitation up to several years. Historically, many of the green sea turtles (*Chelonia mydas*) undergoing rehabilitation were noted to have severely inverted plasma calcium to phosphorus (Ca:P) ratios on routine screening (Stringer et al. 2010).

In the majority of reptile species, the serum calcium value is typically greater than the phosphorus value, and a ratio greater than 1:1 and even approaching 2:1 is commonly considered 'normal'. However, this does not appear to hold for multiple sea turtle species. Published literature reports data that results in calculated low calcium to phosphorus ratios in free ranging wild turtles of several species (Stringer et al. 2010; Stamper et al. 2005) Using published median values for wild green sea turtles off North Carolina, a normal Ca:P ratio for that species appears to be 0.97-1.15 (Snoddy et al 2009, Anderson et al 2011).

Green sea turtles are unique among marine turtles in starting life as carnivores and switching to a primarily herbivorous diet in adulthood. Most rehabilitation facilities, including the KBSTRRC, feed a primarily carnivorous diet to even subadult and adult green sea turtles. Reasons for this include a presumed higher need for protein in debilitated and injured sea turtles, as well as a lack of consistent availability of nutritionally appropriate marine vegetation.

Plasma chemistry panels from green sea turtle patients at the KBSTRRC from 2007 to 2012 were reviewed. From 2007 to 2008, turtles were fed a diet containing a large percentage of squid, which is frequently the preferred food item of animals in rehabilitation. In an attempt to correct the inverted Ca:P ratios, much of this squid was replaced with bluefish for a period of approximately one year. When it was realized that the size of the available bluefish was resulting in only muscle meat being offered, a second diet modification increased the proportion of smaller whole fish fed (including bones and viscera) in the hopes of increasing calcium intake.

This dietary inclusion of smaller whole fish resulted in an increase in Ca:P ratios in the rehabilitated green sea turtles. Samples collected after 30 or more days of rehabilitation from turtles that were being fed large amounts of squid (from 2007-2008) showed a mean Ca:P ratio of 0.533, which tended to decrease during time in rehabilitation, while turtles undergoing rehabilitation when whole small fish were fed (from 2009-2012) had a mean Ca:P ratio of 0.782, with ratios increasing through the rehabilitation period.

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Acknowledgements: I thank Drs. Craig Harms, Betsy Stringer, E.O. (Tres) Clarke, Eric Anderson, Larry J. Minter, Allison Tuttle, Karen Wolf, Sathya Chinnadurai and Greg Lewbart who collected samples over the period studied, and Drs. Craig Harms and Michael Stoskopf for review of the abstract and advice on data interpretation. I also thank Jean Beasley of the KBSTRRC and all of the turtle care volunteers for their dedication to sea turtle rehabilitation and assistance in the collection of research samples.

ELEMENTAL COMPOSITION OF COMMERCIALLY AVAILABLE WHOLE PREY: COMPARISON TO REQUIREMENTS OF DOGS AND CATS

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Whole prey are commonly included in the diets of pet and captive exotic species. However, compositional data for these diet types is lacking. The objective of this study was to evaluate the elemental composition of mammalian and avian species of different ages. Species examined included: mice (1 to 2 d, 10 to 13 d, 21 to 25 d, 30 to 40 d, and 150 to 180 d); rats (1 to 4 d, 10 to 13 d, 21 to 25 d, 33 to 42 d, and > 60 d); rabbits (still born, 30 to 45 d, > 65 d with skin, and >65 d with skin removed); chicken (1 d); and quail (1 d, 21 d, and 35 to 56 d). Additionally, a ground chicken sample and ground duck sample were analyzed.

Composition data on a percent DM basis were compared to AAFCO (2012) recommendations for dogs and cats. These nutrient recommendations are commonly utilized as a model for captive exotic species. Phosphorus concentrations exceeded the safe upper limit of dogs in 21 to 25 d mice; 1 to 4 d, 21 to 25 d and 30 to 40 d rats; 30 to 45 d rabbit with skin removed; and 21 d quail. Calcium concentrations exceeded the safe upper limit for dogs in 21 to 25 d rats, > 65 day rabbits with skin removed, and the ground chicken sample. The Ca:P ratio was less than 1:1 for 1 to 2 d mice and 1 to 4 d rats. Nutrient deficiencies were noted for Mg, Na, K, Cl, Cu, Zn and Mn. For example, Zn was deficient for dogs at all lifestages in all whole prey, and deficient for cats at all lifestages in seven of the whole prey examined.

These data indicate that whole prey should not be considered nutritionally complete foods, and should only be included as part of a properly balanced diet. Additionally, the potential imbalances may have implications for bone health during growth, and species prone to urolithiasis. The high Ca and P levels, and low Ca to P ratio of whole prey are of particular concern for the growth stages of large breed dogs. Further research on the elemental composition of whole prey species is warranted, including the bioavailability and impact on animal physiology (e.g., growth, urine pH etc.) The bioavailability of minerals in whole prey is likely different than those present in processed foods, and may impact the recommendations for feeding.

METANUTRITION: USING METAGENOMICS TO ANALYZE THE GUT MICROBIOME

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The gut microbiome is indispensible for supplementing mammalian nutrition requirements through fiber digestion. Microbial fermentation not only provides short chain fatty acids as a host energy source, but also produces supplemental levels of vitamins B and K. Recent commercial trends toward marketing of probiotic dairy products indicate increased public awareness and interest in promoting desirable bacterial communities, and the medical community has begun to use fecal transplants to restore gut communities after perturbation. But how can we sustain those introduced species? We must feed our microbes to help feed ourselves.

Metagenomics can provide a foundational understanding of microbial communities' metabolic capabilities. Shotgun sequencing targets whole communities, and next generation sequencing enables high coverage sampling at relatively high speeds and low cost. To that end, we have developed MMAP, a bioinformatics pipeline for the streamlined analysis of empirical data. We have combined existing programs and original algorithms to quantify bacterial gene ontology. The functional exploration of high-throughput sequencing data will help characterize gut microbiomes based on their collective strengths, weaknesses, and associations with a specific host or diet. If we use these profiles to infer the microbiota's basic growth patterns and requirements, we can provide the optimal dietary substrate to promote healthy, robust gut populations and optimize digestive performance within the host.

SEVENTH CRISSEY ZOOLOGICAL NUTRITION SYMPOSIUM

Conundrum I

Presented by Dr. Eric van Heugten

A lot of conservation institutions offer their hoofstock (in particular equids, giraffe, camelids, and elephants) emcelle tocopherol. Emcelle tocopherol is a "clear, micellized, water-dispersible, solution of vitamin E." The product is specially formulated for use in liquid diets, mixed into drinking water, or top-dressed onto dry feeds at time of feeding. "

This is a very expensive item, most places offer it daily by body weight = ~$180 per 1L; suggestion is 0.1ml per 50kg BW (although doubling this dose is reportedly common).

Is over supplementation occurring? Are some institutions wasting their money or is there a true need for this product at these levels?

THE POTENTIAL EFFECTS OF DIET AND ENVIRONMENTAL STRESSORS ON CORTISOL RESPONSE IN THE CAPTIVE SOUTHERN RHINOCEROS (*CERATOTHERIUM SIMUM SIMUM)* AT THE NORTH CAROLINA ASHEBORO ZOO

Katie Beth Ellis, BS[1,*], Scott Whisnant, PhD[1], Vivek Fellner, PhD[1], Elizabeth Koutsos, PhD[2], Ryan DeVoe[3], DVM, Kimberly Ange-van Heugten, PhD[1]

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[3] North Carolina Asheboro Zoo, Asheboro, NC

The southern white rhinoceros (*Ceratotherium simum simum*) population at the NC Asheboro Zoo was experiencing difficulties with weight management and perceived high stress levels when confined off exhibit during colder months. Stress being defined as anything that physically or mentally causes a disturbance to allostasis of a given organism. This stress could have radiated affects via the hypothalamic pituitary adrenal axis resulting in suppression of the immune system, digestive malfunction, poor metabolism rates and a decrease in reproductive function (Turner et al., 2002). Seematter et al. (2005) showed that increased cortisol concentrations may lead to visceral fat deposition, with adverse metabolic consequences such as decreasing insulin sensitivity. We were interested in monitoring possible chronic or long term stress markers among the rhinoceroses by means of analyzing fecal cortisol concentrations. Fecal cortisol measurements represent secretion and metabolism over a number of hours and can be quite different than measures of stress from a single moment in time estimate that is provided by blood and salivary cortisol (Whitten et al., 1998a and Whitten et al., 1998b). Results of fecal assays for nutritional digestibility and stress markers, weight loss determinants and blood progesterone levels will determine if nutrition and stress are directly correlated with weight gain and lack of reproductive success in the NC Zoo captive southern white rhinoceros population.

This study monitored fecal cortisol, fecal nutrients and serum progesterone levels in seven (5 females, 2 males) Southern White Rhinoceroses while consuming four different complete feeds over a 6 month period using a repeated Latin Square Design. Fecal samples were taken via direct collection from each rhinoceros for cortisol analysis once a week in the morning from April-December 2012 (n=287 samples, n= 41 samples per rhino) and for nutrient analysis before each diet transition from June through November 2012 (n= 63 samples, n=9 samples per rhino). Collection of fecal samples for cortisol analysis began prior to the first complete feed transition and fecal collection for nutrient analysis in order to establish a fecal cortisol baseline for the herd population. The rhinoceroses were grouped into collection days for the entire 6 month period, Monday collection (2 females, 2 males) and Tuesday collection (3 females). The four complete feeds utilized were ADF 16, ADF 25, Wild Herbivore (WH) and Life Wild Herbivore with Oats (Mazuri, PMI). Complete feeds were randomly allocated to each of the four rhinoceros groups (Table 1) using a repeating Latin Square Model. Each complete feed was administered for a period of 21 days, including five days of transition between the new complete feed and the previous complete feed. Diet transitions from the current complete feed to the new test feed were carried out over the course of five days, allowing each rhinoceros to adjust to the new diet. The series of four complete feed diets was repeated, so that each rhinoceros grouping received each of the complete feeds twice, for a total of 42 days per complete feed during the

duration of the study. Throughout the research period, water and pasture were available ad libitum. Water consumption and pasture consumption amount could not be quantified. The daily amount of complete feed given to each animal remained the same with each new diet administered. Timothy hay was provided (n=1 bale per herd on exhibit, n=10 bales per herd in barn) and one cube of alfalfa or timothy given for training or enrichment on an as needed basis. The rhinoceros population at the NC Asheboro Zoo is pulled off of the free roaming and grazing exhibit during the cold weather winter months, where they are then housed in a heated barn and supplemented with bales of timothy hay in lieu of pasture graze. Fecal samples (0.5g) were extracted with 4.5mL methanol and then dried under nitrogen. Samples were reconstituted in zero cortisol calibrator supplied with the kit and assayed for cortisol using a coated tube radioimmunoassay (Cortisol Coat-a-Count ® Siemens Medical Solution). Preliminary population cortisol results were: μ=0.82 μg/dL, σ=0.45 μg/dL and σ^2=0.21 μg/dL. Current research is underway to quantify differences in fecal nutrients and cortisol concentrations compared to test complete feeds.

Table 1: Latin Square Dietary Rotations for the NC Zoo Rhino (2:5) Groupings Fed Four Different Complete Feeds

	Group 1: Stan & Duma	**Group 2: Kit & Natalie**	**Group 3: Olivia**	**Group 4: Abby & Linda**
Rotation 1A	ADF 16	ADF 25	WH*	LIFE WH (OAT)
Rotation 2A	ADF 25	WH	LIFE WH (OAT)	ADF 16
Rotation 3A	WH	LIFE WH (OAT)	ADF 16	ADF 25
Rotation 4A	LIFE WH (OAT)	ADF 16	ADF 25	WH
Rotation 1B	ADF 16	ADF 25	WH	LIFE WH (OAT)
Rotation 2B	ADF 25	WH	LIFE WH (OAT)	ADF 16
Rotation 3B	WH	LIFE WH (OAT)	ADF 16	ADF 25
Rotation 4B	LIFE WH (OAT)	ADF 16	ADF 25	WH

*WH = Wild Herbivore

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APPLICATION OF NUCLEAR MAGNETIC RESONANCE SPECTROSCOPIC METABONOMICS TO QUESTIONS OF BIVALVE NUTRITION

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Freshwater mussel populations are in decline due to a number of physical and chemical changes to surface waters worldwide. Alterations in food web ecology are likely part of these declines, however, the diet of freshwater mussels is still poorly understood. Lack of knowledge related to nutrition also creates challenges in captive propagation efforts where the goal is optimal reproduction and growth.

Metabolomic (characterizing normal endogenous metabolism) and metabonomic (characterizing metabolic response to a stimulus) investigations can aid in our understanding of nutritional requirements through description of "wild-type" metabolic profiles, isotopic labeling and tracing of carbon sources, and comparison of metabolic responses to variation in feeding practices.

As an example of this process, the metabolic profile, or metabolome, of the freshwater mussel *Elliptio complanata*, and a metabolic perturbation will be described using ^{1}H-nuclear magnetic resonance spectroscopy (NMR) to identify major endogenous metabolites. ^{13}C-NMR suggests that *E. complanata* incorporates carbon from *Bacillus subtilis*, a common soil bacterium, into muscle tissue. Preliminary comparison of fasted *E. complanata* to individuals fed only *B. subtilis* for one week suggests a shift in amino acid metabolism, especially valine/leucine/isoleucine metabolism and glycine/serine/threonine metabolism. Increases in lactic and acetic acids suggest a shift to gluconeogenesis through the catabolism of proteins. Fasted animals showed increased levels of creatine/creatinine pathway metabolites in adductor muscle tissue. Of interest, *Elliptio complanata* has prominent amounts of putrescine, a polyamine, and may use metabolism of putrescine to form glutamate, an important component of amino acid catabolism.

NUTRIENT SUPPLEMENTATION OF AMNION FLUID BY IN OVO FEEDING ENHANCES AVIAN PERINATAL DEVELOPMENT AND POST-HATCH GROWTH

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The perinatal period is critical for development and subsequent growth of avian species because of the high caloric demand to fuel the hatching process, metabolism, and somatic growth until the hatchlings are able to utilize dietary nutrient resources. Maintaining sufficient glycogen reserves is a priority to early survival as the hatchlings make the metabolic and physiological transition from egg to exogenous nutrition. Moreover, in ovo nutrient deficiencies as a result of poor dam nutrition, may further compromise early growth and development of their progeny. To address these nutritional constraints late-term on embryonic development, we developed the in ovo feeding (IOF) technique, which provides late term embryos externally-administered nutrients as they imbibe the amnion fluid prior to pipping. Several experiments were conducted to optimize the IOF supplement formulations that improve energy (glycogen) status, enteric development and digestive capacity, and post-hatch growth. In ovo feeding solution of < 300 mOsm (~10 to 20 μl/g of egg) must be injected into the amnion when the embryo consumes the amniotic fluid (17 to 18 days of incubation for chickens, and 23 to 25 days if incubation for turkeys). At the time of hatch, the degree of enteric development the IOF chicks were observed to be functionally similar to conventional 2-day old chicks offered feed immediately after hatch. Using broiler chicks and turkey poults as the experimental model, IOF increased the digestive capacity of hatchlings as indicated by significantly greater absorptive surface area of gut, increased brush boarder enzyme activities, and greater amino acid and glucose cross-membrane transport activities. IOF also enhanced the expression of genes for brush boarder enzymes, nutrient transporters, and altered the expression of many genes that positively affect energy metabolism and growth. IOF also may enhance the development of humeral and innate immunity. This enhancement in perinatal development by IOF results in significant improvements in post-hatch energy status, growth, and muscle and skeletal development.

Keywords: In ovo feeding; perinatal development; enteric development, gene expression

DIGESTIBLE ENERGY INTAKE AND DIGESTIVE EFFICIENCY OF CAPTIVE NORTH AMERICAN RIVER OTTERS (*LONTRA CANADENSIS*)

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Providing an adequate energy supply is imperative in optimizing the husbandry of captive populations of North American river otters (*Lontra canadensis*). While the metabolic rate of mustelids is considered to be higher than that of other mammals, simply calculating a dietary ration based on the intake of gross energy in the diet can be insufficient.[1] Knowing the digestive efficiency for different captive dietary items is essential because the ingested gross energy cannot be entirely utilized. Diets currently provided to captive North American river otters are highly variable with some institutions providing a diet comprised of various whole food items (e.g., fish, shellfish, rodents, fruit, vegetables, chicken), others feeding commercial complete prepared diets (e.g., Science diet® Feline diet, Topeka, KS; Nebraska Brand® Feline diet North Platte, NE; Iams® Feline diet, Dayton, OH; Mazuri® Polar Bear diet, Saint Paul, MN) and even others providing a mixture of both. The aim of this study was to investigate the digestible energy intake and gastrointestinal transit time, and to determine digestive efficiency of three different primarily fish based diets being fed to the eight North American river otters held at three North Carolina institutions (North Carolina Zoo [NCZ], Asheboro, NC, n = 3 [1.2]; North Carolina Aquarium at Pine Knoll Shores [PKS], Pine Knoll Shores, NC, n = 3 [3.0]; and North Carolina Aquarium on Roanoke Island [RI], Manteo, NC, n = 2 [1.1]). Otters housed at both of the aquariums are fed a majority fish based diet (71-75%), but are supplemented with both fruits, vegetables and novel protein sources (e.g., krill, crayfish claws, shrimp, hard boiled eggs) as enrichment, while the otters housed at the North Carolina Zoo are fed strictly fish. The median digestible energy intake for all eight animals was 163.1 kcal/kgBM$^{0.75}$/day (NCZ, 154.5 kcal/kgBM$^{0.75}$/day; PKS, 228.5 kcal/kgBM$^{0.75}$/day; RI, 144.2 kcal/kgBM$^{0.75}$/day). The differences observed between the different institutions were not statistically significant. Rates of digesta transit were not statistically different between the different institutions, but a trend seemed apparent with transit time being faster in animals fed only fish compared to animals fed fish along with increasing content of vegetables and fruit (NCZ, 106 minutes; PKS, 145 minutes; RI, 208 minutes). Median digestive efficiency was high for all three groups (NCZ, 91.4%; PKS, 87.8%; RI, 89.8%) with no statistical difference observed between the three groups of animals, though an apparent relationship was observed between animals with a higher digestive efficiency and a faster transit time which subsequently were animals fed an only fish diet. Knowledge of energy content, gastrointestinal transit time and apparent digestibility associated with a more fish based diet is important if zoological institutions are to provide a diet which is sufficient for maintenance, growth and reproduction in this highly active species.

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SEVENTH CRISSEY ZOOLOGICAL NUTRITION SYMPOSIUM

Conundrum II

Presented by Mrs. Alejandra McComb

With all we know, How is Metabolic Bone Disease Still a Problem
(even with captive institutions that are “technically doing everything right”)?

As recent papers report, is the lack of natural sunlight the culprit for decreased plasma 25-hydroxyvitamin D3 concentrations?

HEPATIC METABOLOMIC INVESTIGATION OF THE NORTH AMERICAN BLACK BEAR (*URSUS AMERICANUS*) USING ^{1}H-NMR SPECTROSCOPY

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The seasonal variability of metabolism in ursids holds great interest and has been studied extensively. The physiology of hibernation is relevant not only to wildlife biologists, but to scientists studying osteoporosis, renal disease, eating disorders, organ transplant medicine, and a wide array of other fields. We collected hepatic samples from 14 legally, hunter-killed North American Black Bears (*Ursus americanus*) to determine the feasibility and usefulness of high-resolution nuclear magnetic resonance (NMR)-based metabolomics methods in characterizing metabolism in this species. More than 20 major metabolites were identified (Fig. 1), including alkaloids, amino acids, sugars, components of fatty acid metabolism, and Krebs cycle intermediates. The capability of NMR-based metabolomics to detect a wide variety of metabolites illustrates the vast potential of this novel technique for future studies in ursid metabolism.

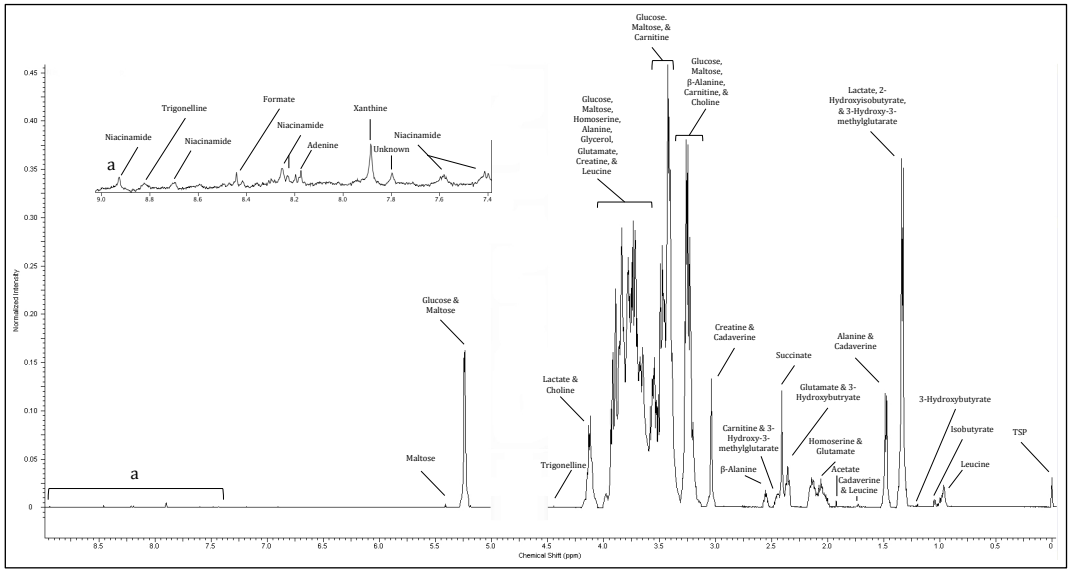

Figure 1. Representative NMR spectrum of the liver of *U. americanus* with major metabolites labeled. The water peak has been removed. The portion of the spectra marked "a" has been enlarged in the inset.

Acknowledgements: The authors thank NC Wildlife Resource Commision wildlife biologists, Colleen Olfenbuttel and Kimberly McCargo for the cooperation in sample collection and support of this project.

EDUCATOR PERSPECTIVES ON MENTORING EXOTIC ANIMAL NUTRITION RESEARCH FOR UNIVERSITY STUDENTS

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Student interest in comparative species research within both the animal science and veterinary science areas increases yearly. In fact, within the NCSU animal science department, entering freshmen survey reports show that they prefer comparative species as their primary and secondary species of interest over all animal categories, except companion animals and equine (Table 1). This statistic is even more interesting since the freshman survey does not have a comparative species choice to pick from and the students have to write it in.

While this exotic animal interest is potentially flattering to those of us within the comparative nutrition field, it ultimately produces many questions without clear answers. Some of the questions to be discussed within this symposium include: 1) How can we select the proper students out of the large numbers of talented and interested students? 2) How can we finance this many students so that they can conduct educational and novel research?; 3) Are we educating more students for a field than there realistically are future career positions and are we being realistic to the students about the potential limitations of the available positions?; 4) How can we develop relationships with conservation centers such that everyone has an obvious benefit from the research (from the animal, to the student, to the keeper, to the establishments, etc.)?; and 5) At what level is the trained student able to call themselves a nutritionist?

Student education loan debt has surpassed credit card debt (over 1 Trillion dollars) as the largest personal debt grouping within the United States and numerous popular articles are being circulated questioning the value of many animal related careers (Martin and Lehren, 2012; Arum and Roksa). Therefore, it is important as educators to make sure that students are being realistic about their education, their career and their finances but it is also important as a researcher and conservationist to assist in the progression of the future of comparative nutrition.

Table 1.Species preferences as indicated by the percentage of entering freshmen who chose each animal as first or second choice, respectively[a].

Animal Category	**Primary Species of Interest**	**Secondary Species of Interest**
Companion Animal	47	23
Horse	30	19
Other = Write In Category (exotic, zoo & marine)	12	20
Beef Cattle	6	7
Dairy Cattle	4	19

Goat	2	5
Sheep	0	2
Swine	0	1
Lab Animal	0	4

[a]Freshman Survey Information provided by Dr. Jeannette Moore (Undergraduate Teaching Coordinator, Department of Animal Science, NCSU).

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Martin, A. and A. W. Lehren. 2012. A Generation Hobbled by College Debt. The New York Times. May 13, 2012.

MANUFACTURING FEEDS FOR EXOTIC ANIMALS

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General principles of feed manufacturing are applied to produce feeds for exotic animals. Sourcing and purchasing ingredients (commodity and specialty ingredients) involves a number of considerations, including price, nutrient composition, and an understanding of normal variation of those ingredients. Formulation of exotic animal feeds often uses similar computer programming tools as those used in commercial livestock diet formulation programs, but can be based on least cost formulation, fixed formulation, or constant nutrition. Formulae may be manufactured into many forms, with limitations based on equipment, ingredients, and finished product specifications (e.g., a pelleted diet will not float while an extruded diet may float depending on manufacturing conditions). The extent of finished product testing will be dependent on the application and on cost considerations (nutritional analyses can add considerable expense to a feed).

The feed industry is regulated by a number of agencies, via policies such as the establishment of uniform feed ingredient definitions and proper labeling to assure the safe use of feeds. The Food and Drug Administration (FDA) regulates animal feed under the Food, Drug and Cosmetic Act and has inspection authority in manufacturing facilities. State Departments of Agriculture regulate the manufacture and distribution of commercial feed within their state, and also have inspection authority in manufacturing facilities. The Association of American Feed Control Officials (AAFCO) creates clarity and uniformity among state and federal feed control rules and regulations.

The use of medication in animal feeds is a routine request, and is regulated by the federal government. The Minor Use and Minor Species Animal Health Act of 2004 legislation governs the ability to provide medication for minor uses and minor species (defined as all animals except humans, cattle, horses, swine, chickens, turkeys, dogs and cats). At this time, there are a very limited number of approved drugs for exotic animals. FDA has issued a Compliance Policy Guide (615.115) for Extra-Label Use of Medicated Feeds For Minor Species, although there are conditions that must be satisfied in order to use: 1) Veterinarian involvement; 2) Treatment only use; 3) No production use; and, 4) No feed reformulation or re-labeling. Feed must be manufactured and labeled as approved for use in a major species. Again, this results in an inability to produce medicated diets for exotic animals. Further, the risk of cross-contamination of other feeds with drugs that may be toxic or harmful to species for which the drug is not intended provides further impetus for cautious use of medications in the feed manufacturing facility.

In summary, manufacturing quality feed is a highly complex process governed by federal and state regulations, and driven by internal and external quality assurance processes.

METABOLIC CONSEQUENCES OF THE EARLY ONSET OF OBESITY IN COMMON MARMOSET MONKEYS

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Introduction

We previously reported on changes in lean and fat mass in marmoset infants followed from birth until one year of age (Power et al., 2012). About half of the subjects were classified as Fat at 12 months of age (percent body fat greater than 14%). We now report on measures of glucose metabolism (fasting glucose and insulin) in these marmoset infants at 6 and 12 months of age, comparing Normal infants (percent body fat less than 14%) to Fat infants. We also report on circulating leptin and adiponectin at these ages. Finally we present the results of oral glucose tolerance tests (OGTT) in a subset of these animals at 12 months of age. We test the hypotheses that animals with excess adipose tissue will display higher circulating leptin, lower circulating adiponectin, higher fasting glucose, decreased insulin sensitivity, and decreased ability to clear glucose from the blood stream.

Methods

Percent body fat was measured by quantitative magnetic resonance. Circulating glucose was measured using a glucometer. Insulin, adiponenctin and leptin were measured at the Assay Services Division if the Wisconsin National Primate Research Center. The QUICKI, a measure of insulin sensitivity, was calculated for all subjects with both fasting glucose and insulin.

Blood samples matched with body composition measurements were collected from 33 subjects at 6 months of age, resulting in values for blood glucose for 32 subjects, leptin for 31 subjects, insulin for 11 subjects and adiponectin for only 3 subjects. At one year of age blood samples matched with body composition measurements were collected from 39 subjects, resulting in values for blood glucose for all 39 subjects, leptin for 37 subjects, insulin for 23 subjects and adiponectin for 29 subjects.

Results

At 6 months of age Fat subjects tended to have higher fasting glucose ($p=.063$) and did have significantly lower insulin sensitivity (mean QUICKI = .378±.029 versus .525±.019, $p=.003$). By 12 months of age Fat subjects had both significantly higher fasting glucose (129.3±9.1 mg/dL versus 106.1±6.5 mg/dL, $p=.042$) and lower QUICKI (.317±.010 versus .513±.018, $p<.001$). Circulating adiponectin tended to be lower in Fat subjects at 12 months as well ($p=.057$). Leptin was associated with percent body fat at 12 months of age, and was greater in Fat subjects (Table 1). However, birth weight appeared to exert an additional influence on circulating leptin levels.

The OGTT results demonstrated that Fat animals had poorer glucose control than did normal subjects (Figure 1). Fat subjects had a faster rise in blood glucose, with significantly higher values at 15 minutes and 30 minutes post dose. Blood glucose for both fat and normal subjects peaked at 60 minutes at about 1.7 times fasting levels with no significant difference between the groups. By 120 minutes normal animals had returned to baseline glucose values, but fat animals had significantly higher glucose values, about 1.3 times baseline on average.

Discussion

We have previously described evidence of metabolic dysfunction in adult marmosets that were above the 80th percentile in the proportion of body fat (Tardif et al., 2009). We have also documented that obesity in young marmosets occurs as early as 6 months of age, and that on average animals that are above 14% body fat at one year of age already had higher body fat as early as one month of age (Power et al., 2012). These data demonstrate that marmosets classified as obese as juveniles (12 months of age with 14% body fat or greater) showed clear signs of impaired insulin sensitivity, including higher fasting blood glucose, elevated fasting insulin levels, and a tendency to have lower circulating adiponectin. When subjected to an oral glucose tolerance test Obese subjects had a more rapid rise in blood glucose and elevated blood glucose at 120 minutes, a time point when Normal animals had returned to baseline blood glucose levels. These results taken together indicate that the early onset of obesity has significant negative metabolic consequences for one-year old marmosets, with animals above 14% in body fat displaying evidence of a reduction in insulin sensitivity and greater difficulty maintaining glucose homeostasis. The more limited data from 6 month old marmosets suggests that this pattern is already beginning to be established at that early age.

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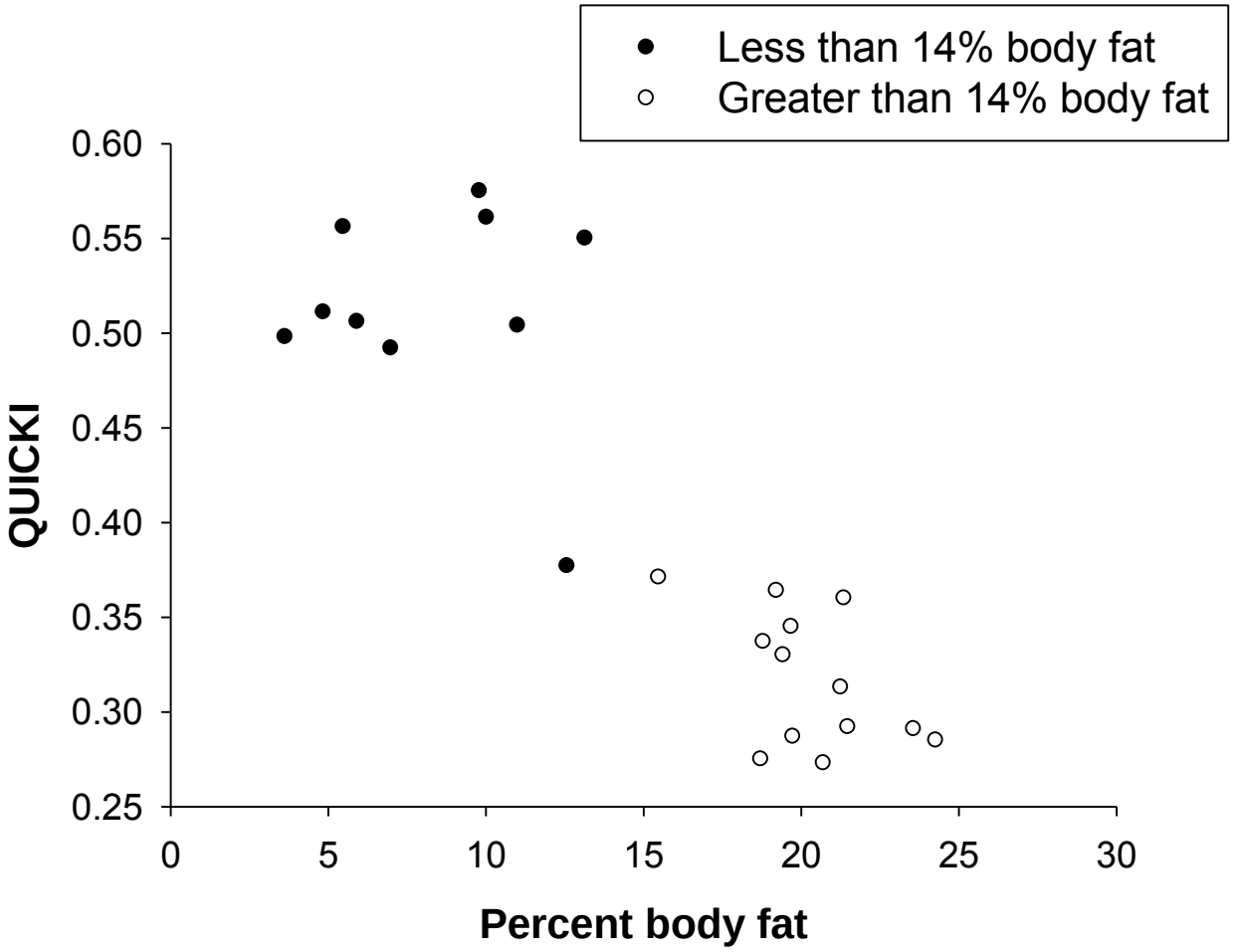
Table 1. Insulin, glucose and leptin were higher in animals with above 14% body fat at one year of age.

	Normal animals	Fat animals	P value
Glucose	106.1±6.5 mg/dL N = 21	129.3±9.1 mg/dL N = 18	.042
Insulin	1.01±.25 µU/ml N = 10	16.45±3.04 µU/ml N = 13	<.001
QUICKI	.513±.018 N = 10	.317±.010 N = 13	<.001
Leptin	.73±.09 ng/ml N = 19	1.18±.15 ng/ml N = 18	.014
Adiponectin	9.83±1.54 µg/ml N = 14	6.53±.71 µg/ml N = 15	.057
High molecular weight Adiponectin	1.10±.24 µg/ml N = 14	.80±.13 µg/ml N = 14	.294

Fig 1. QUICKI versus body fat at one year of age



Ossabaw and Commercial Swine as Models for Juvenile Obesity Research

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The alarming increase in childhood obesity over the last 30 years has resulted in an explosion of research geared toward understanding, prevention and intervention. Obese children are at greater risk for cardiovascular disease factors, such as high blood pressure and high cholesterol. They are more susceptible to development of insulin resistance, leading to type II diabetes. They also suffer from psychological stress in the form of low self-esteem and depression [(1)]. While much of the research to date has been performed in rodent species, a more appropriate model is needed. Swine are an attractive model for this type of work for several physiological reasons including body and organ size, cardiovascular anatomy, bone remodeling system, and adipose cell size and distribution [(2)]. The purpose of this research is to define an appropriate swine model for childhood obesity.

Ossabaw swine were isolated on an island off the coast of Georgia, in the United States, for around 500 years. During this time, they developed a "thrifty genotype," allowing them to consume excessively to survive seasons of feast and famine. For this reason, they are capable of developing insulin resistance, impaired glucose tolerance, hypertriglyceridemia and hypercholesterolemia when fed obesigenic diets [(3)], making them an excellent model for obesity and its impact on cardiovascular diseases. We examined the effects of an obesigenic diet on bone integrity and on mesenchymal stem cells (MSC) activity, which underpins bone integrity. Ossabaw pigs fed an obesigenic diet had greater ($P < 0.05$) body fat % and insulin resistance. Obese pigs had larger and heavier bones ($P < 0.05$), however the percentage of mineral in the bone did not differ between obese and lean pigs. In order to evaluate alterations in MSC activity, MSC from obese and lean pigs were cultured under adipocytic and osteogenic conditions. Under osteogenic conditions, the expression of genes indicative of osteoblastic differentiation (RunX2 and osteocalcin) were greater ($P<0.1$) in MSC isolated from obese pigs. Under adipogenic conditions, increased gene expression of markers of adipocytic differentiation (PPARG, AP2, and LPL), as well as greater lipid accumulation (Oil Red O staining), were also seen in obese pigs. Gene expression and ORO staining suggest that obese MSC may be acting as more mature cells, differentiating more readily into both fat and bone cells upon induction with treatment medias. These results suggest that obesity and insulin resistance may have long-term effects on bone development and MSC activity.

Rapid growth during infancy has been associated with increased risk of childhood obesity. We examined the effects of increased growth rate caused by overfeeding on growth, bone integrity, and activity of MSC in commercial neonatal piglets. Limit fed (LF) animals received a standard piglet milk replacer at a rate to achieve normal growth, while the overfed (OF) group voluntarily consumed nearly 60% more of the same diet for 21 days. Overfed piglets had higher growth rate ($P < 0.001$) and feed intake ($P < 0.05$), and lower feed efficiency ($P < 0.001$) compared to LF piglets. The OF animals had heavier, larger bones with greater bone mineral content than the LF group ($P < 0.05$). Surprisingly, MSC isolated from OF animals

adopted an adipocytic lineage less readily than those isolated from LF animals after induction with adipocytic media. This was indicated by lower gene expression of PPARG, LPL and AP2 ($P < 0.05$) as well as by decreased lipid accumulation determined by Oil Red O staining. Increased ($P < 0.2$) gene expression of Runx2 following osteogenic induction was also seen in MSC from OF pigs. Accelerated growth during infancy led to a decreased response of MSC to an adipocytic environment, as well as an increased response to osteogenic induction. These results indicate the potential for neonatal dietary interventions to influence growth and body composition later in life via programming of MSC.

Due to the large prevalence of obesity within monogastric zoo species and its impact on other health issues, including reproduction, research using swine could positively influence animal nutrition practices in numerous captive zoological environments.

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[3.] Dyson MC, Alloosh M, Vuchetich JP, Mokelke EA, Sturek M. 2006. Components of Metabolic Syndrome and Coronary Artery Desease in Female Ossabaw Swine Fed Excess Atherogenic Diet. Comparative Medicine. 56:35-45.

TRANSCUTANEOUS RUMP ULTRASOUND OF ASIAN ELEPHANTS (*ELEPHAS MAXIMUS*): BODY FAT, BODY CONDITION AND BODY WEIGHT

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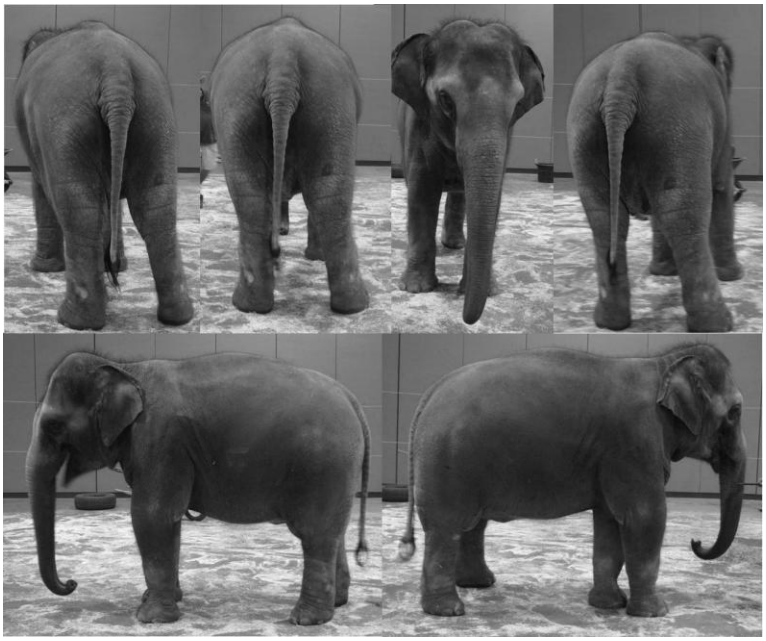
Body condition is a risk factor in multiple disease states including cardiovascular disease, metabolic dysfunction, foot and joint issues and reduced reproductive success. In order to evaluate the impact body condition may have on the health and sustainability of elephants, a standardized, precise and accurate body condition scoring is required. Visual body condition scoring (BCS) systems have been published based on elephants in Asia[1-3], but no visual body condition scoring system has been validated by direct measures of fat. Total body fat has been shown to correlate closely to ultrasonic measure of rump fat in cattle[4], horses[5], cervids[6-9] and dogs[10]. This study presents the first BCS method for elephants correlated to ultrasonic measures of subcutaneous rump fat.

MATERIALS AND METHODS

Twelve Asian elephants (*Elephas maximus*) from 3 institutions in North America were sampled concurrently for visual body condition and transcutaneous rump ultrasound. Four elephants at the Fort Worth Zoo were sampled longitudinally for evaluation of body condition changes within individuals.

Visual body condition: Visual body condition was assessed from a standardized photoset including front (for identification), both sides, direct rear and 45° rear (both sides) (Figure 1). For all photographs the animal was standing relaxed and with feet square. Photosets were scored by 3 trained scorers using a 9 pt scale developed at the Fort Worth Zoo based on over 100 standardized photosets of captive Asian elephants and numerous individual photos of captive and wild Asian elephants in North America and Asia. This visual scale corresponds well to published BCS scales for Asian

Figure 1. Standardized photoset for body condition scoring.



elephants[1-3], with notable differences being enhanced detail (scoring 9 degrees of fatness rather than 3 or 5) and exclusion of the head as an area of consistent fat deposition.

Ultrasound: Transcutaneous ultrasounds were collected using an ALOKA SSD-500V and UST-5049-3.5Mhz linear probe recording 18 cm in length and 19 cm in depth. Ultrasound locations were brushed clean of dirt and hay and sprayed with 70% isopropyl alcohol before applying the probe with generous ultrasound gel. Location was selected based on clarity of image, anatomical landmarks and consistency of fat layer. The ultrasound was placed on a vertical line parallel to the tailhead 50% of the distance between the tailhead and the tuber coxae (Figure 2). Two images were collected per side: one beginning with the head of the probe at the top of the pelvis (tuber sacrale) and the second lower but with images overlapping slightly. Digital ultrasound images were measured by 3 trained scorers for fat layer (not including skin) at the middle of the 2 images (MID), the widest area of fat (MAX) and from the bottom of the skin to the visible pelvis bone at the middle of the image (PEL) (Figure 4). Muscle thickness was calculated as the difference between MID and PEL.

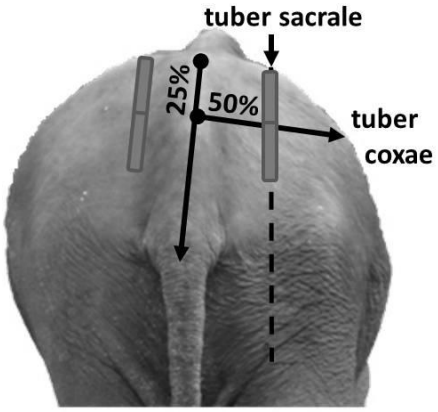


Figure 2. Standardized transcutaneous rump ultrasound. Measurements are made 25% from top of the pelvis (tuber sacrale) to rectum and across 50% to the outer iliac spine (tuber coxae). Grey rectangles represent overlapping probe locations.

Statistics: Variables were compared by simple linear regression. Significance was defined as $P > 0.05$. Scaling of fat thickness (MID) to body size was performed by dividing MID by estimated surface area[9,11], body weight (BW), metabolic body weight (BW^0.75) and frame size (height x BW).

RESULTS

MID, Muscle and PEL showed significant moderate correlation with visual BCS (Table 1, Figure 3), but muscle thickness was not correlated to fat thickness ($p = 0.12$). Scaling of MID to body size did not improve correlation coefficients. Interquartile range for the residuals was -0.7 to 0.9 BCS scores for MID and -0.6 to 0.4 BCS for PEL. Longitudinal measures showed strong correlation ($r > 0.929$) of BCS to MID, PEL, muscle and bodyweight in one mature animal with 3 BCS

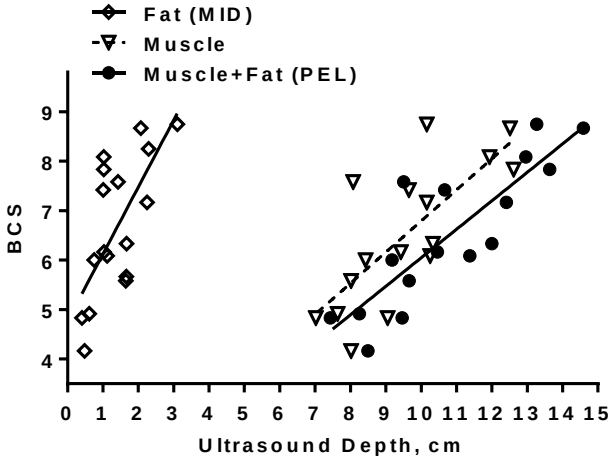


Figure 3. Correlation between rump ultrasound fat thickness (MID) and muscle plus fat thickness (PEL) and body condition score (BCS) in Asian Elephants.

scores ranging from 5.6 to 8.8, but only to MID and PEL in one growing animal with 3 BCS scores from 4.9 to 7.6.

Table 1. Descriptive statistics of Asian Elephant subjects (n=12).

	Bodyweight, kg	BCS of 9	MID, cm
Median (range)	3200 (1491-4242)	6.25 (4.17 - 8.75)	1.07 (0.41-3.11)
	Regression	p-value	r
MID	BCS = 1.35(MID) + 4.77	0.001	0.705
Muscle	BCS = 0.63(Muscle) + 0.50	0.001	0.746
PEL	BCS = 0.58(PEL) + 0.29	<0.001	0.849

DISCUSSION

This study is the first to document the relationship between a direct measure of fat (rump ultrasound) and a subjective visual body condition scoring system in Asian elephants. BCS predicted MID within ±0.5 cm and PEL within ±1.0 cm based on the variation of the residuals. Inversely, MID predicted elephant BCS within ±1 score and PEL just over ±0.5 scores.

According to the relationship observed between BCS and measured tissue layers, elephants accumulate muscle on the rump from approximately BCS 1 to 4.77 then begin laying down 0.74 cm fat and 1.59 cm of muscle depth per BCS

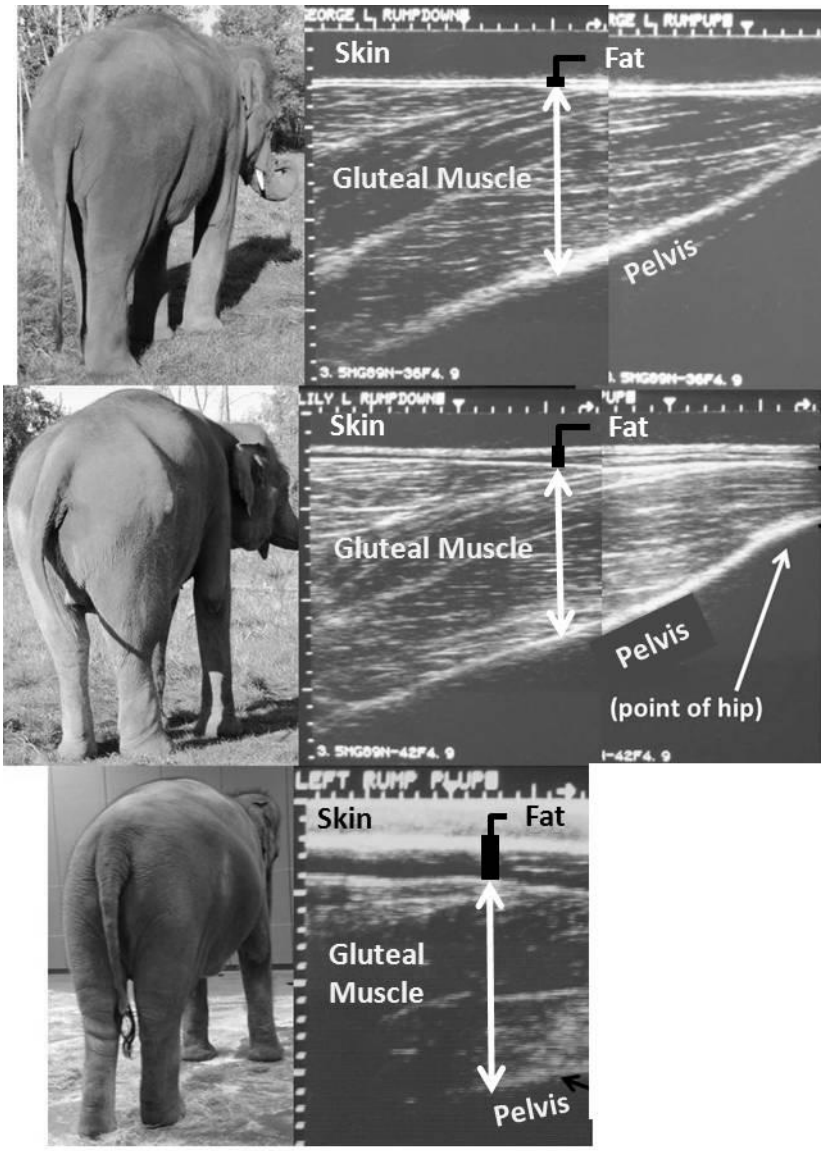


Figure 4.Asian elephant rump ultrasounds representing a body condition (BCS) range of 4.8 to 8.7.

increase above 5. In other species rump fat depletion has been observed to occur between BCS of approximately 2 to 4.5 and correlates to a median total body fat (TBF) of 8% (herbivore range 5-11%) (Table 2). The elephant value of 4.77 may be slightly overestimated by the data as fat was still observed in all elephants, including one scoring 4.17 BCS (Figure 4).
Other notable areas of bias are the poorer resolution/precision of elephant images due to greater total image depth, the fact skin was removed from the elephant depth measure, and the use of MAX fat thickness in cervid species[6-9]. In the present study, MAX was only different from MID in three elephants and did not show any consistent pattern in location or with BCS independent of MID so was dropped from analysis. This difference from cervid species studies may represent variation in how species deposit fat, degree of fatness across species, or ultrasound image location.

The rump thickness observed in elephants of high BCS (~3 cm) was comparable to the highest rump thickness reported for other herbivore species ranging from 50 to 550 kg bodyweight, but substantially higher than observed rump fat thickness of dogs and raccoons (<30 kg) (Table 2). This suggests that scaling of fat thickness does occur, but may account for less variability in animals greater than 50 kg than interspecies differences in fat storage.

Table 2. Comparisons of rump fat measures, total body fat (%TBF) and body condition scores (BCS) normalized to a 9 pt scale in species of varying bodyweight.

		Change per 1 cm rump fat			Point of rump fat depletion		Rump fat	Change per 1 BCS
Species[1]	BW, kg	%TBF	kg TBF	BCS	%TBF	BCS	Max cm	%TBF
Raccoon	4			15.0		2	0.75	
Cat	4							6.6
Dog	25	97.7	19.5	15.9[2]	17.5	4.25[2]	0.5	6.3
Mule deer	40	5.9	2.4		5.1			
Sika deer	45						3.5	
Sheep	73							3.3
Caribou	100	2.6	2.6	2.3[2]	8.1	4.5[2]	3	1.5
Pony	150	3.8	8.4		5.6		3	
Red Deer	180						5	
Elk	188	3.6	6.8	1.5	5.5	3.75	3.7	2.4
Horse	480	4.2	20.2	2.4	10.7	3	3.5	1.7
Moose	500	2.1	10.2	0.75	5.8	2.12	7	2.7
Cow	550	8.5	46.8	2.2	9.0	2.5	3.5	4.3
Elephant	3200	2.6[b]	83[c]	1.35		4.77	3.1	1.9[a]

[a] Numbers based on individual changes in weight and BCS.
[b]Calculated from the BCS change/cm rump fat and %TBF change/BCS
[c]Calculated from the %TBF change and average bodyweight
[1]Data adapted from studies in raccoon[12], cats[13], dogs[10,13-15], mule deer[9], sika deer[16], sheep[17], caribou[6,18], pony[5], red deer[19], elk[7], horse[5], moose[8], cow[4]. All BCS scales were normalized to 9 pt based on 5 being moderate, 1 minimum and 9 maximum fatness.
[2]Values estimated from multiple papers in the same species

The elephant is unique due to its size and could provide invaluable information on the scaling of fat deposition across bodyweight. Cook et al. attempted to determine scaling factors for rump thickness in 3 species of cervids ranging from 40 to 500 kg[9]. The elephants in the present study alone ranged over 2500 kg, yet attempts to scale MID by weight, metabolic bodyweight, surface area[11], or frame size failed to account for individual variability. Similarly rump fat thickness across species ranging from 4 to 3200 kg (Table 2) showed only weak relationships to body mass with high variation presumed to be attributable to interspecies differences in preferred patterns of fat deposition (e.g. subcutaneous versus internal fat or rump versus ribs) or differences in BCS systems. A rough estimate of %TBF per increase in BCS across species can be calculated as $10 \cdot BW^{-0.21}$ (Figure 5). This would predict a change of ~5.1% TBF per BCS score in 25 kg animals, ~2.7% TBF per BCS score in 500 kg species and ~1.8% TBF per BCS score in elephants.

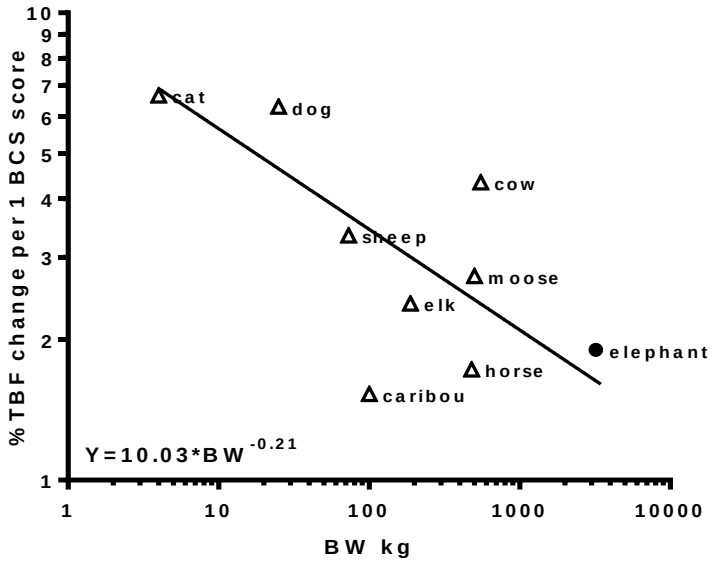


Figure 5. Change in total body fat (%TBF) per 1 body condition score (BCS) across species of varying body

For the 2 elephants weighed and scored longitudinally in this study (discarding 1 data point due to growth), 1 BCS score was associated with a 200 kg change in total bodyweight (~6% BW). If the proportion of fat in this gain is estimated to be 32% from the proportional change in MID and Muscle - the change in fat would be 63 kg, or ~1.9% BW, which is close to the 1.6% TBF gain predicted by the scaling equation. One cm of elephant rump fat thickness equated to a change in 1.35 BCS, therefore an estimated change of 2.6% TBF (83 kg).

The Association of Zoos and Aquariums recently suggested that elephants be managed between a BCS of 6 and 10 on the 11 pt Wemmer scale[3,20]. These values correspond to a 4 to 7 on our 9 pt BCS scale for Asian elephants (personal communication), a range matching recommendations for domestic species. This range would correspond to 0-1.65 cm rump fat depth and potentially 7-12% TBF (224-384 kg) in Asian elephants.

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SEVENTH CRISSEY ZOOLOGICAL NUTRITION SYMPOSIUM

Conundrum III

Presented by Dr. Shannon Pratt-Phillips

Now that we are capable of using NMR techniques to quantify metabolites and conduct metabolomic studies, we are naturally looking at ways to process samples from animals in the wild. As that becomes feasible, how can we best deal with the problem that we will be trying to do sophisticated metabolic assessments on animals when we have no idea of what they have eaten prior to our non-lethal sampling?

DEVELOPMENT OF METHODS FOR ASSESSING GREAT APE BODY FATNESS

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Introduction

Obesity in captive apes is a perceived problem, which could put them at risk for obesity-related diseases observed in humans (1,2). Overweight and obesity is determined in humans by standardized methods including indirect measures such as body mass index and skinfold measurements, and direct measurements such as total body water and dual x-ray absorptometry (3). Recently, ultrasound measurements of subcutaneous adipose tissue have been validated as a method for accurately estimating total body fat in young adults (4,5). Standardized, validated tools for assessing body fatness do not exist for non-human primates, but they are needed in order to accurately investigate target body conditions and condition-related health risks in these species.

Methods

Measures were obtained from four individuals from three great ape species (1.0 orangutan (*Pongo pygmaeus*), age 39; 1.1 chimpanzee (*Pan troglodytes*), ages 23 and 37; 0.1 bonobo (*Pan paniscus*), age 17) during physical exams. Animals were palpated at focal areas (abdomen, buttock, thigh, tricep, scapula, chest, ribs) by two-to-three trained researchers, weighed, and measured for skinfolds using a standard caliper (orangutan, 1.0 chimpanzee) (Table 1). Transcutaneous ultrasounds of multiple sites (6-10 locations per animal, including chest, rib, scapula, bicep, tricep, thigh, abdomen) were taken using a Titan SonoSite ultrasound machine with a L52/10-5 MHz linear probe. Hair was parted at ultrasound locations and exposed skin was sprayed generously with 70% isopropyl alcohol before applying the probe with ultrasound gel. Ultrasounds were collected by placing the probe in the following locations: abdomen (2 cm laterally to either side of the umbilicus); upper pelvic girdle (horizontally on the lower back directly above the upper pelvic bone); front thigh (in line with the femur halfway between the hip and knee); rear thigh (in line with the femur halfway between the buttock and knee); scapula (medial to the center of the scapula, parallel to the spine); chest (lateral to the nipple); upper rib (lateral to the middle sternum); lower rib (lateral to the bottom of the sternum, halfway across the ventral rib cage); bicep (in line with the center of the biceps brachii); tricep (in line with the humerus, halfway between the elbow and shoulder). The same areas were not measured for all animals due to positioning of the animal and/or time constraints.

Due to poor delineation between skin and fat layers in the ultrasound images, subcutaneous skin-adipose thickness (SSAT) was used as an indicator for subcutaneous adipose thickness (SAT). SSAT was measured from the middle of the ultrasound images, except for the abdomen where it was measured 2 cm laterally from the umbilicus. An example ultrasound image is provided in Figure 1. All animals were considered to be in moderate condition (neither under or overweight) based on palpation and observation during physical exam and discussion with experienced animal managers.

Results
Skinfold thickness measures in the orangutan and male chimpanzee showed remarkable agreement with the ultrasound SSAT measures ($y=0.9631x + 0.0086$, $r=0.97$) (Figure 2). The areas of greatest skin-adipose thickness were the abdomen, thigh, tricep, and forearm, with the chest and bicep having less skin-adipose thickness (Table 1). Necropsy revealed bias in some areas due to skin thickness (i.e. forearm had no fat).

Discussion
Ultrasound SSAT measures were compared across the four animals to examine patterns of fat deposition. Abdomen, thigh, and chest SSAT appeared to increase proportionally across the four animals (Figure 3). Upper pelvic girdle, scapula, and rib SSAT did not vary in the same pattern. Individual variation in fat deposition and skin thickness could be due to differences in body shape, species, or gender. Also, some measures (e.g. ribs) require more standardization for true comparison.

In humans, abdominal SAT is highly correlated with percent body fat (% BF) in both men ($\rho=0.907$) and women ($\rho=0.905$) (5). One population of normal to obese subjects showed men to have a median abdominal SAT of 1.39 cm (range 0.1-5.73 cm) and median % BF of 17.5 (range 7.8-34.5), while women had median abdominal SAT of 2.82 cm (range 1.01-7.4 cm) and median % BF of 31.1 (range 16.3-52.1). The abdominal SSAT measures (0.78-1.93 cm) obtained for the four apes in this study fall at the lower end of the measured abdominal SAT ranges observed in the human subjects, and are overestimated due to inclusion of the skin layer. If smaller abdominal SAT measures reported in the human subjects correspond to the lower (moderate) end of the reported % BF range, the abdominal SSATs of these four apes close to the low end of the measured SAT range in human subjects support our assessment of their moderate body fatness. Visual assessment of primates is subjective and can be challenging due to body hair and conformation, making alternative methods of evaluating obesity such as abdominal ultrasound attractive and possibly essential. Training an ape to participate in an awake ultrasound should be possible and likely easier than attempting skinfold measures. Validation of abdominal fat as a proxy of total body fat in great apes is still required.

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Table 1. Morphometric and ultrasound measures of four great apes at the Fort Worth Zoo. Abbreviations: SF, skinfold; US, ultrasound.

	Orangutan 1.0				**Bonobo 0.1**	**Chimpanzee 1.0**			**Chimpanzee 0.1**
Condition	Moderate-low				Moderate	Moderate			Moderate-high
Weight (kg)	102.97				49	82.2			66.68
	Median (Range) Skin+Adipose Thickness (cm)								
Location	**SF (L)**	**SF (R)**	**US (L)**	**US (R)**	**US (R)**	**SF**	**US (L)**	**US (R)**	**US (L)**
Thigh, front	1.4	1.5	0.59 (0.55-0.62)	0.64 (0.56-0.72)			0.74	0.75	
Thigh, rear	1.3	1.4			0.36 (0.34-0.39)		0.76	0.99	0.91
Upper pelvic girdle		1.1-1.2			0.68 (0.61-0.75)		1.34		1.09 (1.07-1.10)
Scapula					0.64 (0.58-0.70)		0.78		0.76
Abdomen	2.4	2.2	1.23 (1.18-1.29)	1.08 (1.03-1.10)	0.78	2.85 (L) 2.8 (R)	1.46	1.54	1.93
Rib, upper								0.87	
Rib, lower			0.60 (0.47-0.65)	0.65 (0.57-0.65)	0.39 (0.36-0.39)		1.06 (1.02-1.09)	1.44	1.01 (0.92-1.22)
Chest	0.7	0.7	0.42	0.34	0.42 (0.34-0.47)			0.62	0.71
Tricep	1.15-1.3	1.3	0.72 (0.68-0.76)	0.80 (0.56-1.00)	0.93 (0.93-0.94)			0.91	1.68
Bicep	0.85	0.7	0.45	0.46	0.38			0.44	

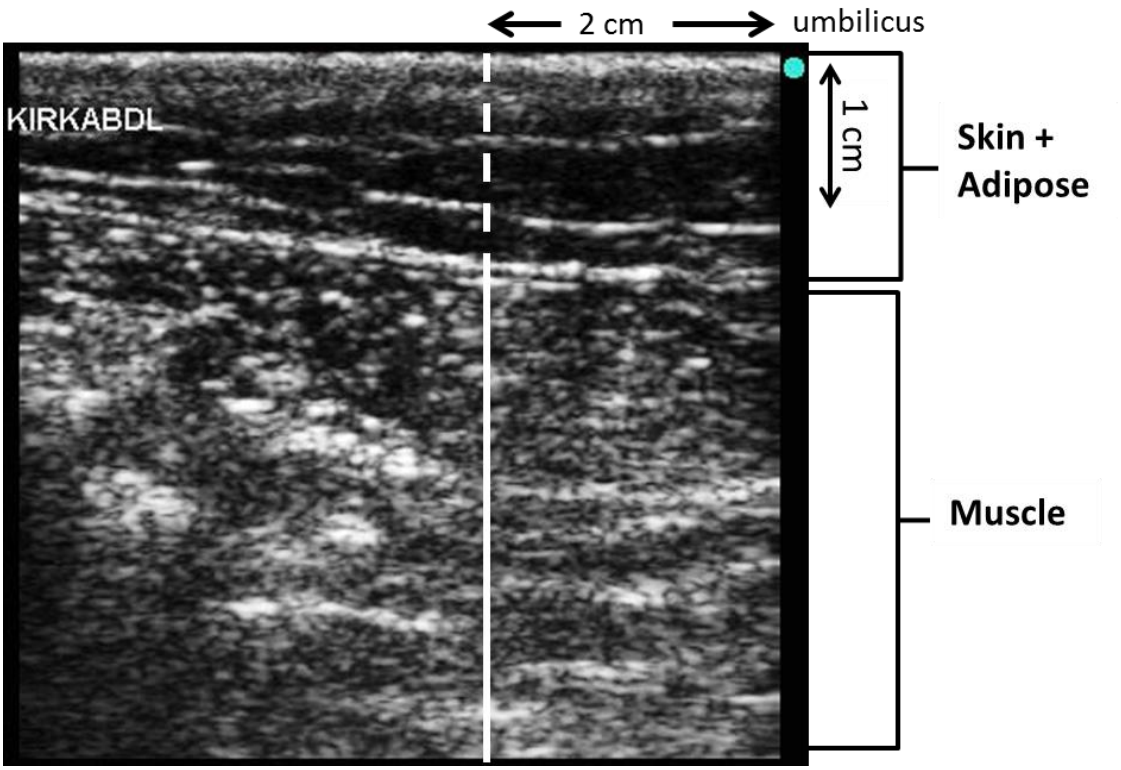

Figure 1. Example ultrasound image of the abdomen (adjacent to the umbilicus) of a male chimpanzee from the Fort Worth Zoo showing the subcutaneous skin-to-adipose (dashed line) and muscle (solid line) layers.

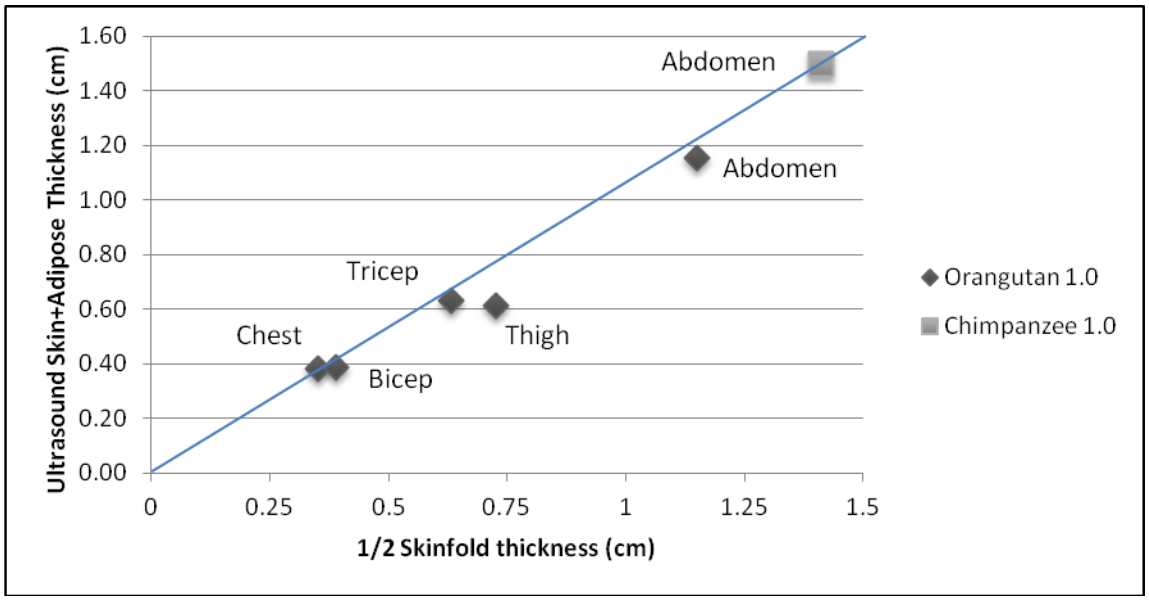

Figure 2. Relationship between measured skinfold thickness and ultrasound subcutaneous skin-adipose thickness (SSAT) at different sites in four great apes. Linear slope represents line of identity (1:1).

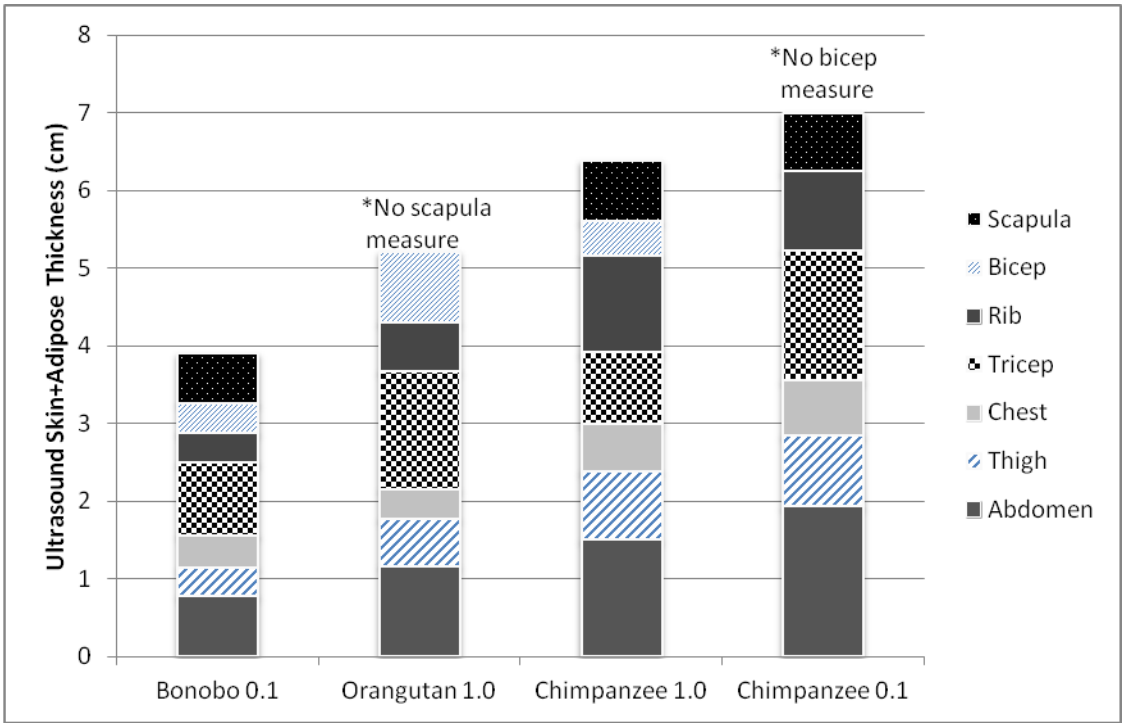

Figure 3. Comparison of ultrasound subcutaneous skin-adipose thickness (SSAT) at different sites in four great apes.

HAND-REARING MONK PARROTS (*MYIOPSITTA MONACHUS*)

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Introduction

There is limited information and research establishing the maintenance and growing energy requirements of parrots by species. The majority of available research on energy requirements in avians has focused on poultry, especially chickens, as well as budgerigars. The energy requirements for poultry can have limited application to hand-rearing parrots due to poultry species being precocial and raised for maximal production purposes whereas parrots are altricial with longevity and health being the future goals. It may be possible to estimate growth energy needs from the adult maintenance energy requirements as done with dogs and domestic cats (SDCN, 2006).

Hand-raising birds is commonly performed by pet bird breeders, zoological institutions, and rescue/rehabilition facilities, who establish individualized methods over years of experience and previous success. This methodology, however, does not ensure the birds will achieve optimal health as adults. While the established methods result in the birds living to adulthood, they may lead to detrimental phenotypic effects as adults. Additionally, they may result in excess body fat in weanlings. Many chronic, progressive inflammatory diseases, including atherosclerosis, are linked to excess body fat. To help improve bird health, it is important to determine the energy requirements of adult and growing birds of many species and additionally ensure public access to this information.

Methods

Feral Monk parrots ranging in age from embryos to early fledglings were donated to the Schubot Exotic Bird Health Center at Texas A&M University during late Spring and Summer, 2011. Previously hand-reared chicks (in 2010) experienced periods of delayed followed by catch up growth. In an attempt to prevent potential health issues from fluctuations in growth rates experienced by the birds hand-reared in 2010, energy requirements of growing Monk parrots were estimated and a protocol was established for hand-rearing the chicks (Protocol-2011, Table 1). The energy requirements of growing Monk parrots were estimated by doubling a previously published existence energy requirement equation (kcal per day) of non-passerine birds (0.5404*W^0.7545, W=g) (Kendeigh, 1970) and one for mammals (110*W^0.75, W=kg) (SDCN, 2006). A similar strategy has been recommended for small mammals including growing canine species (SDCN 2006). The birds were divided into four smaller groups consisting of 3-8 birds based on weight and stage of development at time of acquisition.

Results and Discussion

Data was obtained from 18 hand-reared Monk parrots. The growth curve for the average of all chicks hand-reared in 2011 closely followed that of reported parent-reared chicks (Caccamise and Alexandro, 1976) up to approximately day 30 post-hatching. Two sub-groups diverged to a slower growth rate around 20 days of age. The other two sub-groups closely followed the growth curve of the parent-reared Monk parrots (Caccamise and Alexandro, 1976) up to 30 days of age.

Analysis of the energy intake of these later two sub-groups revealed that these birds received more kcals per day than Protocol-2011 called for beginning around day 20. Thus, it can be concluded that the 2-fold increase of the adult existence energy equation for non-passerines (Kendeigh, 1970) originally estimated as the chicks' energy needs per day was lower than their actual requirements after day 20. When Adult Monk parrots maintaining weight and housed at the Schubot Exotic Bird Health Center were observed, they were found to consume 1.87 times more kcals energy per day than estimated by the Kendeigh (1970) equation.

Three different periods of energy intake were noted in the 2011 hand-reared chicks. Between day 0 and 18 of life, the chicks consumed on average 1.02*W^0.7545 (W = g) kcals per day, the same as the adult Monk parrot maintenance energy requirement. During day 18 to 23 of growth, the hand-reared chicks consumed 1.43*W^0.7545 (W = g) kcals per day or 1.4 times adult Monk parrot maintenance energy requirements on average. After day 23, it was observed that the chicks' total energy intake per day remained relatively constant, but the chicks continued to increase their body weight. This suggests a decrease in the energy requirements of the chicks occurs after 23 days of life.

Ricklefs (1967) determined a graphical method of determining the K constant for use in comparing growth rates between avian species. The K constant for parent-reared Monk parrots is reported to be K=0.1624 (Caccamise and Alexandro, 1976). Following the same method, the K constant for the average of the chicks hand-reared in 2011 was determined to be K=0.1676, and thus similar to the parent-reared chicks.

The similar K constant found for the birds hand-raised in 2011 with those that were parent-reared suggests that the 2011 hand-raised birds grew at an acceptable rate. This also suggests that the energy consumed by the 2011 hand-reared birds is similar to the amount parents provide to their chicks. Thus, our Protocol-2011 (1.02*Wg^0.7545 kcal per day) provided adequate energy through day 18 (~54 g body weight). However, due to increased energy needs for feather development and growth between days 18-23 Monk parrot chicks should be provided 1.4*Wg^0.7545 kcal per day during this time.

Acknowledgements: Schubot Exotic Bird Health Center at Texas A&M University; Rick Jordan of Hill Country Aviaries; Hatch Team and volunteers who helped feed the Monk parrots

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Table 1. Feeding protocol for 2011 hand-reared Monk parrots. Commercially available diets were used for this protocol. Feed dilution and number of feedings were calculated to meet the estimated energy requirements of the growing Monk parrots using the estimated calories of the chosen commercial diets. Volume per feeding was estimated from percent of body weight that can be safely fed to baby birds. Times are listed in 24-hour clock format.

Age (days)	Body Weight (grams)	Volume Per Feeding	Formula to Water (kcals/ml)	Feeding Times
0-4	5-9	0.3-0.7 ml	1 part formula A:3 parts water (1.0 kcal/ml)	3, 6, 8, 10, 12, 14, 16, 18, 20, 22, midnight
5-8	10-15	0.5-1 ml	1 part formula A: 2 parts water (1.3 kcal/ml)	6, 8, 12, 14, 16, 18, 20, 22, midnight
9-21	20-50	2-5 ml	1 part formula B: 3 parts water (0.82 kcal/ml)	6, 9, 12, 15, 19, 22
22-40	60-80	6-8 ml	1 part formula B: 2 parts water (1.1 kcal/ml)	6, 10, 14, 18, (22)
41-60	90-120	6-10 ml	1 part formula B: 2 parts water (1.1 kcal/ml)	6, 10, 14, (20) offer weaning foods

EFFECTS OF PROCESSING TIME ON BLOOD AND PLASMA SAMPLES FROM LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) FOR ^{1}H-NMR-BASED METABOLOMICS

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The biological end products of metabolism are known to change immediately after a sample is collected from a living animal. Given the high sensitivity of nuclear magnetic resonance (NMR)-based metabolomics, efficient sample handling and processing becomes a concern for accuracy and reproducibility of results. We collected heparinized whole blood samples from 5 rehabilitated loggerhead sea turtles (*Caretta caretta*). An initial aliquot of whole blood was immediately frozen on dry ice. Additional aliquots were held at ambient temperature, centrifuged, and then plasma was frozen after 15-, 30-, 45-, and 60-minutes after the blood was collected. More than 10 metabolites were identified in the resulting ^{1}H-NMR spectra with a greater peak diversity seen in the whole blood samples. The spectral changes in plasma samples over time were minor (Fig. 1). These results confirm whole blood as a practical and biologically appropriate metabolomic sample in this species and also indicate that blood collected from turtles up to 1-hour post-mortem would still be expected to be valuable for metabolomic study.

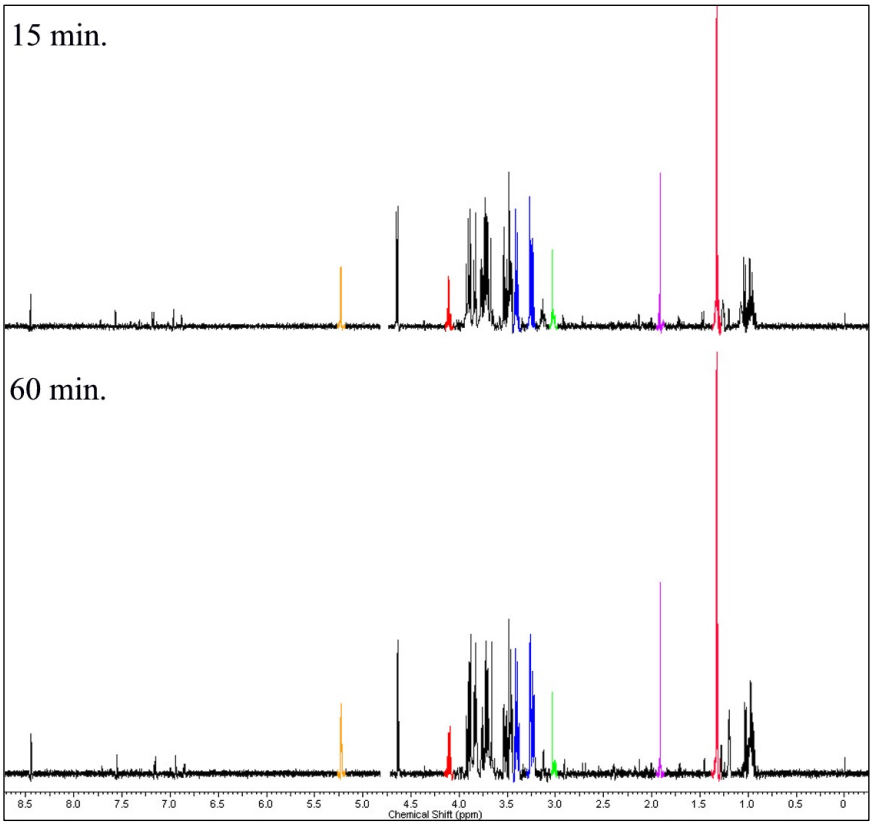

Figure 1. Comparison of representative NMR spectra of plasma samples from *C. caretta* after 15 and 60 minutes. The water peaks have been removed. Red = lactate, purple = acetate, green = creatinine, blue = taurine, and orange = one of the peaks for glucose.

Acknowledgements: The authors thank Jean Beasley and the Karen Beasley Sea Turtle Rescue and Rehabilitation Center for the cooperation in sample collection and support of this study.

EFFECT OF REDUCED ANIMAL-BASED PROTEIN AND TOTAL PROTEIN IN THE CAPTIVE DIET OF MANED WOLF

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Introduction

The captive management of maned wolves in US zoos has proven to be challenging. Numerous, prevalent ailments have been appreciated in the population including cystinuria, renal calculi, inflammatory bowel disease, wasting, and poor body condition.

Of particular concern is cystinuria and renal calculi, which seem to arise with greater incidence in this species than would be expected based on occurrence in the domestic dog. This has caused death in maned wolves from complications due to stones. There is some evidence that this occurs in both captive and wild populations, though the data from wild animals is limited both by individuals and geographic locations sampled. It should be noted that heredity has not been ruled out as a contributing factor.

Perhaps predisposing to this issue is that, despite being generalist omnivores, maned wolves are typically fed as carnivores in captivity. Many captive diets rely heavily on animal-based items and may therefore contain much higher levels of cystine than this species would encounter naturally. Cystine can acidify the urine, creating an environment conducive to the development of crystals and renal calculi. Commonly, urine-alkalizing agents such as potassium citrate are supplemented in the diet to diminish the formation of urinary crystals. However, cystine itself could be limited in the diet and reduce the occurrence naturally.

In anticipation of a necessary diet change due to feed availability, steps were taken to formulate a new diet at Dickerson Park Zoo that might more appropriately sustain this species with respect to cystinuria. The maintenance diet for these animals consisted almost completely of animal-based items. The purpose of this study was to determine if the reduction of animal-based protein and total protein in maned wolf diets could maintain or improve animal health, while reducing the heavy reliance on potassium citrate for control of cystinuria.

Methods

Five maned wolves (3.2) at Dickerson Park Zoo participated in this study in 2008 and 2009. Animals ranged in age from 2-12 years old. Two trials were conducted for each animal, separated by a several month wash-out period after the diet transition.
Diets offered in each trial can be found in Table 1. The trial 1 diet consisted of the animals' maintenance diet at that time. The trial 2 diet was formulated to have lower protein from animal sources, as well as lower protein overall. Fat was increased slightly to keep both diets iso-caloric as much as possible. Expected differences of the two diets were determined by entering food item nutrient levels into Zootrition 2.6 and analyzing total diet nutrient composition (Table 1).

Table 1. Ingredients and estimated ME, crude protein, crude fat, and crude fiber of trial diets fed to maned wolves at Dickerson Park Zoo in 2008 and 2009.

	Original Diet (Trial 1): 320g: Exclusive chicken and rice dry dog food 200g: Natural Balance 10 50g: Apple 70g: Banana		Lower Protein Diet (Trial 2): 250g: Bil-Jac senior dry dog food 85g: Natural Balance 10 17g: Mice 400g: Romaine lettuce 135g: Apple 110g: Banana 165g: Tomato 30g: Canola oil	
Nutrients (DM)*				
ME (kcal)/g	4.14	74% from dog food 19% from NB 10 6% from produce	4.2	56% from dog food 10% from NB 10/mice 34 % from produce/oil
% Crude Protein	30.06	70% from dog food 29% from NB 10 1% from produce	20.72	65% from dog food 22% from NB 10/mice 13% from produce/oil
% Crude Fat	16.03	69% from dog food 30% form NB 10 1% from produce	19.36	42% from dog food 12% from NB 10/mice 46% from produce/oil
% Crude Fiber	Analysis in progress but expected some increase with change to more produce			

*values calculated via Zootrition 2.6

Trials were conducted for 5 days with total fecal and ort collection. Animals were weighed before and after each trial. Urinalysis was conducted when a fresh, clean urine sample was available. Fecal quality was assessed using the Waltham fecal scoring system for domestic dogs. Banked blood samples existed for 3 of the 5 animals from before and after diet changes. Two animals only have blood samples from pre-diet change as they were transferred to a different facility soon after their second trial. Serum chemistry values may provide further insight into changes potentially associated with the diet change.

All food offered and orts were weighed to determine feed intake. Desiccation pans were set out in similar conditions to the diet pans, protected from animals and pests, to determine any change in moisture due to evaporation or humidity for uneaten portions. Orts and samples of diet items were also collected for analysis of diet offered vs. diet consumed. For each animal, dry matter was determined for each fecal sample. Individual fecals were then combined for each trial and a sample taken for nutrient analysis. All samples will be analyzed for dry matter, gross energy, crude protein, crude fat, ash, ADF, NDF, Ca, P Mg, K, Na, Fe, Mn, Cu, Zn, and S.

Results of nutrition analysis will be compared statistically to illuminate any differences.

Results

We expect our results to show that while the source and level of protein may differ between the two diets, animals were able to be well-maintained on the lower protein diet. We also expect to see a rise in urine pH owing to the lower level of animal based protein. Should a rise in urine pH occur the next step would be to reduce the reliance on potassium citrate as a urine-alkalizing agent.

Animal weights did not change significantly from their average when transitioned to the lower protein diet, though some animals did lose weight once on this diet. However, subjectively, animals maintained good body condition through and beyond the diet transition as well as showing improvements to coat condition on the lower protein diet. Some animals did show minor fecal quality improvement, while others did not. Ideal fecal scores fall between 1.5 and 2.5 (on a 1-5 scale). Animals in this study averaged 3-3.5 in trial 1 and 3.0 in trial 2. Overall, it appears fecal quality was not significantly affected though the trend leans towards better.

We expect serum chemistry values for both trials to align within the expected ranges for this and other canine species. Values will be analyzed for significant differences between trials.

Discussion

The results of this study will likely only shed light on a small part of issues seen in captive maned wolf populations. The expectation is that the diet changes reflect a movement towards more appropriate nutrition in captivity based on wild-type diet composition and shown by improved health of captive individuals. However, there are many other factors that may play a role in management of cystinuria and other diseases in maned wolves.

Going forward, chemical analysis of diets in nutrition trials should include amino acid composition as cystine in particular has proven to be of concern. Also, while this study focused on digestibility via fecal collection, urine should also be examined. Unfortunately, we were not in a position to collect urine for more than opportunistic urinalysis. Additionally, other factors can affect the development of urinary crystals including water consumption and moisture content of the diet. Research exploring this may also contribute to better overall animal health and improvements to diets.

As mentioned before, heredity of this condition has not been ruled out as a contributing factor. This disease process shows similarities to the genetic disorder found in domestic dogs and humans. Why it seems to appear at a higher rate in maned wolves is unknown. It is known that the majority of captive-held animals, as well as wild animals tested for cystinuria do originate from the same geographical location. Therefore, it is possible that conclusions drawn from these animals should not be applied to the maned wolf species as a whole.

Wild maned wolves can and do rely heavily on small mammal and bird prey items for much of the year, during the dry season. While analysis of wild diet items is lacking, one could hypothesize that amino acid composition is comparable to other animal-based food items. There may be other environmental or dietary factors allowing wild animals to manage the occurrence naturally to which captive individuals would not be exposed. Therefore, it would be prudent to explore the genetic etiology of this disease within the wild population as compared to the captive population as well as a greater understanding of wild diet composition.

Furthermore, while diet changes and medical interventions can be made for the management of this disease, it is important to know whether this disease is limited to the captive population (and therefore managed as an isolated genetically-related population) or if maned wolves in general have a higher predisposition to this disease. With the population listed as near threatened and expectations that wild numbers will continue to decline, perpetuation of such an ailment in the captive population could prove disastrous should wild populations ever need to be buttressed with captive-reared animals.

METABOLOMIC INVESTIGATION OF HATCHLING LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) USING ^{1}H-NMR SPECTROSCOPY

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Upon hatching, sea turtles must be metabolically ready for the impending frenzy when the nest is opens and the hatchlings "boil" out and head to sea where they swim long distances. To evaluate the energetics of this stage of sea turtle life, we collected cardiac and skeletal muscle samples from 8 hatchling loggerhead sea turtles (*Caretta caretta*) and whole blood, hepatic, and bile samples from 3 of the hatchlings. The samples were analyzed using high-resolution nuclear magnetic resonance (NMR)-based spectroscopy to establish baseline metabolic profiles and to determine the advantages and disadvantages of using different tissue metabolomes to assess hatchling sea turtle condition. More than 50 metabolites were identified in the tissues sampled. Principal components analysis (PCA) demonstrated distinct characteristics of each tissue. Blood samples were characterized by high content of myo-Inositol and glucose. Cardiac muscle was differentiated by high glutathione concentrations, whereas carnosine was more abundant in skeletal muscle. Hepatic tissue had high levels of the sugars glucose and maltose. Over all, whole blood provided a complex but readable spectrum that we determined to be a practical and biologically appropriate to serve as a sample for metabolomic studies in this species (Fig. 1). Baseline metabolomic information obtained from these studies can be applied to future hatchling loggerhead studies focusing on the effects of incubation temperature and post-hatching activity.

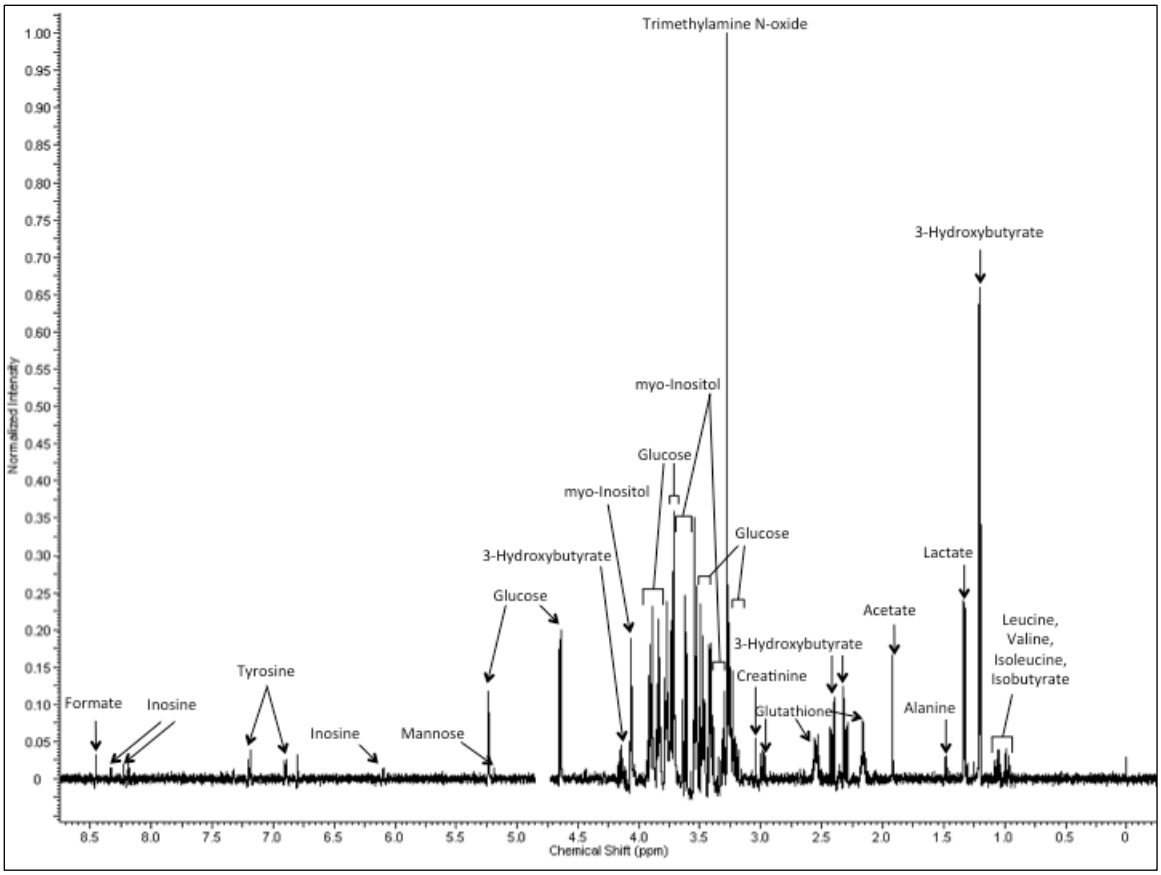

Figure 1. Whole blood spectrum with major metabolites labeled. The water peak has been removed.

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COMPARISON OF PROXIMATE COMPOSITION OF DOMESTIC CAT (*FELIS CATUS*), CLOUDED LEOPARD (*NEOFELIS NEBULOSA*), AND AFRICAN LION (*PANTHERA LEO*) MATERNAL MILK WITH EXOTIC CAT HAND-REARING FORMULAE

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Introduction

The ability to produce milk is a trait unique to mammalian mothers. Milk acts as an external pathway of communication between mother and offspring, continuing the vital exchange of nutrients that was previously facilitated in utero by the placenta. The composition of maternal milk directly impacts the development trajectory of a nursing offspring as any milk constituent deficiencies could result in developmental pathologies such as nutritional cataracts (Cooley, 2001) and inadequate or inappropriate growth. Thus it is important to recognize that even amongst closely related species, differences in lactation strategies often produce differences in milk composition.

Animals born in captivity sometimes require nutritional intervention if maternal milk is not an option. In such a situation, the knowledge of that species' milk composition is integral in producing comparable milk replacer formulae. The domestic cat (*Felis catus*), a well-studied species in the commercial industry, is typically used as a model for exotic felids (Bell et al., 2011). Commercial hand-rearing formulae developed for the domestic cat are therefore commonly employed for captive exotic felids. However, little is known about the actual maternal milk composition of exotic felids and how it compares to the composition of hand-rearing formulae.

The aims of this study are (1) to compare proximate composition of milks of three felids, the domestic cat (*Felis catus*), clouded leopard (*Neofelis nebulosa*) and African lion (*Panthera leo*) to establish if there exist species-specific differences in felid maternal milk composition, (2) to conduct proximate analyses on three commercial hand-rearing formulae commonly used by zoological institutions for clouded leopards and African lions, (3) to compare the maternal milk compositions of the before-mentioned felids against the compositions of the selected hand-rearing formulae, and (4) to provide hand-rearing formulae recommendations to the zoological community for clouded leopards and African lions.

Methods

Proximate analyses of milk and formula samples will be performed at the Smithsonian Conservation Biology Institute's Nutrition Lab. A survey will be distributed through the Nutritional Advisory Group forum asking zoo professionals willing to assist in this study to provide their clouded leopard and African lion hand-rearing formulae and recipes. Three hand-rearing formulae will be selected from the survey responses and subsequently replicated and analyzed in the Smithsonian Conservation Biology Institute's Nutrition Lab. Proximate analyses on KMR® (Kitten Milk Replacer), a commonly used commercial hand-rearing formulae used for

exotic felids (Grant, 2005) and one that is routinely employed by the National Zoo, have already been completed (Table 1).

Results and Discussion

Our preliminary results from the proximate analyses of KMR® liquid formulae agree with those of Edwards and Hawes, 1997 (Table 1). Additionally, we expect our results for the proximate composition of domestic cat milk to reflect the well-established averages for domestic cats reported in Jacobsen et al., 2004 (Table 1). From the domestic cat milk and KMR® liquid formula results illustrated in Table 1 we note a discernible difference in the average fat content of domestic cat milk to that of KMR® liquid formula as well as a slightly higher crude protein value in milk versus the formula. Fat and protein content of milk are associated with body growth rates for developing offspring (Hinde and Milligan, 2011) and disparities in these nutrients between maternal milk and hand-rearing formulae could account for the slower growth rates sometimes observed in hand-reared felids (Grant, 2005).

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Table 1. As-fed basis comparison of the maternal milk composition of the domestic cat and KMR® liquid formula. Jacobsen et al. (2004)[1]; Edwards and Hawes (1997)[2]; Murtough, Power, and Maslanka (2012)[3].

	Domestic Cat[1]	KMR® Liquid [2]	KMR® Liquid [3]
Dry Matter, %	27.9	19.30	18.51
Crude Protein, %	8.7	7.70	7.58
Fat, %	12.7	4.68	4.68
Carbohydrate, %	4.2	4.74	4.74
Ash, %	1.3	1.18	1.59

HOW DO YOU MILK A GORILLA? INTRODUCTION TO THE MILK REPOSITORY AT THE SMITHSONIAN CONSERVATION BIOLOGY INSTITUTE VIA A PRACTICAL EXAMPLE

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Lactation is a defining characteristic of mammals. All female mammals produce milk from glandular mammary tissue that their offspring ingest. The diversity of the mammalian radiation is reflected in the diversity of lactation strategies that have evolved. This in turn is reflected in differences in milk composition among mammals. All milks contain the necessary nutrients for existence; however, the proportions vary widely. Milk is a complex biological fluid. Its study is an important, but relatively neglected component for understanding the evolution of mammals.

The milk collection at the Smithsonian Conservation Biology Institute (SCBI) represents a unique research resource. The Nutrition Laboratory at SCBI has extensive experience in assaying milks; milks from over 200 species have been assayed, including over 30 species of primates. Validated techniques for accurately measuring the proximate nutrient composition of milk (dry matter, fat, protein, sugar, minerals, and energy) have been established at the lab. Proximate analysis can be accomplished on as little as 0.5 ml of primate milk. A large number of milk samples from a wide variety of mammals are stored frozen.

New samples continue to be collected from zoos, research colonies, and wild animals. In addition, published data from previously assayed samples are available in summary form, as well as some unpublished data. Three doctoral dissertations on primate lactation have been facilitated by the laboratory and the milk collection to date. Any milk samples available are welcomed for submission into the Repository. Based on needs, sample results can be provided in a timely fashion for management decisions or larger-scale research projects. Researchers interested in coming to the SCBI Milk Repository to learn assay techniques and gain assistance with the processing of their samples and interpretation of their data can be accommodated.

Most recently, through a collaboration between the Department of Nutrition Science and the Primate Department at the National Zoological Park, milk from a lactating female gorilla (*Gorilla gorilla*) was collected from parturition through 31 months of lactation to establish a longitudinal assessment of milk composition changes. Changes in dry matter, protein, fat, and sugar were assessed over that period of time in a novel project facilitated by the Milk Repository and training skills of the animal care staff at the National Zoological Park. Results of the longitudinal assessment will be presented in a poster format, as well as a video presentation of the actual process of milk sample collection.

ACCURACY OF ESTIMATING GROSS ENERGY OF MILK FROM VARIOUS SPECIES

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Introduction

Energy is required for proper growth and development of young animals. However, the energy composition of milk for many species is unknown. Furthermore, the direct determination of energy usually requires a large sample volume, which is difficult to acquire from many species. One solution to this problem is to estimate the energy composition of a milk sample based on other constituents in the milk. Previous studies focused on determining which nutrients were the most important for accurately estimating gross energy and their conversion factors in cow milk (Overman and Sanmann, 1926; Overman and Gaines, 1933). A few studies have also been completed in other domestic animals with estimated energy being up to 5% higher than determined values when not corrected for non-protein nitrogen (Perrin 1958).

Methods

Milk samples were part of the Smithsonian National Zoological Park's Milk Repository and had been stored at -20°C since collection. For this study 7 White Rhinoceros (*Ceratotherium simum*) samples from 2 individuals, 5 Black Rhinoceros (*Diceros bicornis*) samples from 2 individuals, 10 Rhesus Macaque (*Macaca mulatta*) samples from 10 individuals (Hinde et al., 2009), and 13 Bongo (*Tragelaphus eurycerus*) samples from 2 individuals were analyzed.

Samples were assayed for dry matter (DM), fat, sugar, crude protein (CP), and gross energy using standard methods that have been validated at the Nutrition Laboratory of the Smithsonian National Zoological Park and performed on milks from about 200 species of mammals. Briefly, for DM, milk samples were aliquoted, weighed, and dried in a forced air convection drying oven for 3.5 hours at 100ºC and then reweighed (AOAC, 1990). Total nitrogen (TN) was determined for the dried milk samples using a carbon, hydrogen, and nitrogen (CHN) elemental gas analyzer (Model 2400, Perkin Elmer, Norwalk, CT). This method has been validated against the macro Kjeldahl procedure with nitrogen recovery around 98-99%, and has been used at Smithsonian National Zoological Park to measure milk nitrogen for a wide variety of species. The obtained nitrogen value was multiplied by 6.38 to determine the amount of CP in the milk. Total lipid was measured using a micro modification of the Roese – Gottlieb procedure by means of sequential extractions with ethyl alchohol, diethyl ether, and petroleum ether. Total sugar was analyzed by the phenol – sulphuric acid colorimetric procedure (Dubois et al., 1956; Marier & Boulet, 1959) using ultraviolet spectroscopy and lactose monohydrate standards.

Gross energy (GE) content of the milk was determined by adiabatic bomb calorimetry. Samples of milk were freeze-dried and then pelleted or rolled into a compact circle. Values were corrected for heat formed by the combusted fusewire. Gross energy was also calculated using the formula: 9.11 * fat + 3.95 * sugar + 5.86 * CP. The conversion factors in this formula were suggested by Anderson (1926) and agreed upon (except for an added corrective factor for non-protein nitrogen) by Perrin (1958).

Results and Discussion
The samples ranged in fat from 0.2-25.2%, protein 1.1-13.1%, and sugar 2.7-8.2%. Thus a large range of milk compositions were analyzed in this study.

When comparing determined gross energy values to estimated gross energy values, it was found that better correlations were obtained when using values on an As-Is basis rather than a dry matter basis.

Milk samples for all species tested had significant correlations between determined and estimated gross energy (Table 1). There were no significant differences between determined gross energy and estimated gross energy for any species analyzed when compared with a paired t-test (Table 1).

There are no significant differences in the gross energy value obtained for a milk sample whether it is determined by adiabatic bomb calorimetry or estimated using the equation 9.11 * fat + 3.95 * sugar + 5.86 * CP. While Perrin (1958) advised accounting for non-protein nitrogen fractions, which differ between species, the good correlations obtained without this correction in this study suggests the correction does not significantly affect the gross energy estimation and is not necessary. Thus, the formula 9.11 * fat + 3.95 * sugar + 5.86 * CP could be used when trying to determine the average gross energy value of milk for a species needing to be hand-raised.

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Table 1. Statistical analysis of estimated gross energy versus calculated gross energy.

		Correlation		Paired t-test	
Species	**Number**	**r**	**P-value**	**t**	**P-value**
Bongo	n = 13, N = 2	0.972	<0.0001	-1.702	0.115
Rhesus Macaque	n = 10, N =	0.991	<0.0001	1.387	0.199
White Rhinoceros	n = 7, N = 2	0.936	<0.002	0.204	0.845
Black Rhinoceros	n = 5, N = 2	0.952	<0.001	0.993	0.377
Rhinoceros	n = 12, N = 4	0.996	<0.0001	0.836	0.421

DUSTING CRICKETS TO SUPPLEMENT WITH RETINOL AND CALCIUM: EFFECTIVENESS AND DURATION

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INTRODUCTION

Crickets are a commonly fed to amphibians in captivity as a sole source of food, but are naturally low in calcium and vitamin A,[1] putting amphibians at risk for metabolic bone disease[2,3] and squamous metaplasia.[3] To date, nutritional requirements of amphibians have not been determined, therefore targets levels are based primarily on carnivore requirements[4] due to the assumption that the amphibians in question consume only animal matter. Target calcium level may be defined as 0.95% Ca DM based on levels required to avoid metabolic bone disease in leopard geckos (*Eublepharis macularius*).[2] Target retinol levels may be defined as 47 ppm DM based on an oral dose given to Puerto Rican crested toads (*Bufo lemur*) maintained at the Fort Worth Zoo without signs of vitamin A deficiency and squamous metaplasia.[5]

Gut loading and dusting are common practices for supplementing crickets with additional calcium and vitamin A but no complete or dependable supplementation method exists.[5-8] Crickets groom themselves and shed their skins, therefore the effectiveness of dust depends largely on how quickly the crickets are consumed.[9] The objective of this study was to evaluate cricket dusting supplements to determine how long target levels of calcium and vitamin A could be achieved in dusted crickets.

MATERIALS AND METHODS

Half-grown crickets (*Acheta domestica*) were obtained from a commercial supplier (Timberline Live Pet Food, Marion, IL) and maintained for 1 day in large bins with egg crates, water trays and sliced apple. On each day of the study a baseline sample (40g crickets) was collected at ~7 am and immediately placed in a freezer at -20°C. Test samples were collected into gallon size ziplock bags and gently shaken with 1 tsp of supplement powder: either 50% calcium carbonate + 50% Nekton Rep vitamin/mineral supplement (Dust A; Günter Enderle NEKTON produkte, Germany), Repashy Vitamin A Plus (Dust C), or Repashy Calcium Plus (Dust B) (Repashy Ventures, Inc., San Marcos, CA) in order to completely coat the outside of the crickets. The dusted crickets were then transferred to a screen in order to sift excess dust before being placed in a ventilated terrarium with egg crates and water trays and stored in a room (79-84°F) with the lights turned off. Terrariums were transferred to the freezer 0, 1, 6, or 24-hours after post dusting. After at least 3-4 hours in the freezer, frozen crickets were carefully transferred to a coffee grinder cup, weighed, then ground to a homogenous one millimeter. Ground samples were placed in a light protected container and stored at -80°C until analyzed. Sampling was repeated on 4 different weeks, with each week representing 1 replicate of each time x dust combination.

Lab Analysis: Ground cricket samples were analyzed at the Fort Worth Zoo Nutritional Services Laboratory, Fort Worth, TX for vitamin A content according to previously described methods.[5,10]

Dry samples were sent to Dairy One Forage Lab (Dairy One Cooperative, Inc. Ithaca, NY) to determine calcium concentrations according to AOAC methods.[11]
Statistics: Calcium and log transformation of retinol concentrations were analyzed by repeated measures ANOVA with tukey-corrected multiple comparisons. Data are reported as mean ± standard deviation unless indicated. Significance was defined as $P < 0.05$.

RESULTS

Baseline concentrations of supplements and crickets are listed in Table 1. Results for dusted crickets are shown in Figure 1. Baseline crickets weighed 0.08 ± 0.11, 0.10 ± 0.33, 0.07 ± 0.01, 0.07 ± 0.01 g for replicates 1-4 respectively. There was no correlation between sample cricket size and calcium or retinol concentrations ($P < 0.05$). Cricket skins were observed after 1, 6, and 24 hours. The majority of sheds were observed at 24 hours and represented roughly 5% of crickets.

Table 1. Retinol and calcium (dry matter basis) concentrations of dusting supplements and baseline crickets.

	50% Nekton Rep + 50% CaCO3 (Dust A)	Repashy Calcium Plus (Dust B)	Repashy Vitamin A Plus (Dust C)	Baseline Crickets
Retinol (ppm)	856	152	1622	Not Detectable
Calcium (%)	20.31	23.45	18.92	0.09%

Cricket calcium concentration was higher at all time points for Dust B ($P<0.05$) than Dust A and C but never different between Dust A and C ($P>0.05$). Calcium concentration dropped at each consecutive time point for all dust treatments ($P<0.05$). All cricket samples had calcium concentrations greater than 0.95% DM (dry matter).

Cricket retinol concentration was lower ($P<0.05$) at all time points for Dust B than Dust A and C but never significantly different between Dust A and C ($P>0.05$). Retinol concentrations dropped between 1 and 6 hours for all dust treatments ($P<0.05$). Average retinol concentration was greater than 47 ppm only at 0 and 1 hour post dusting with Dust A and C.

DISCUSSION

In this study, retinol was not detected and calcium levels were low for baseline crickets suggesting the need to supplement these nutrients for animals consuming crickets as their main source of food. All samples for all time periods were above the target level for calcium, indicating that dusting, in agreement with other studies, may be a successful method for supplementing crickets with calcium.[7,8] Under normal conditions Ca is absorbed according to need and excess is excreted.[12] Max tolerance levels for Ca are 2% for cattle, sheep, horses and rabbits, 1% for swine, 1.2% for most poultry and 4% for laying hens.[12] Higher calcium levels can be associated with poor palatability, growth abnormality and could impact availability of other nutrients.[12] The calcium level of crickets in this study reached almost 7%; it is unknown whether such high levels are a concern in amphibians.

In the present study, as in other similar studies, dusting showed limited success for supplementing crickets with adequate vitamin A.[8] Between 1 and 6 hours, vitamin A concentrations dropped below target nutrient levels, presumably due to the crickets grooming themselves as shedding was not a significant occurrence before 6 hours. Dusted crickets would provide adequate vitamin A only if consumed within the first hour after dusting. The supplements used in this study were not different from typical dusting supplements which can vary significantly in retinol (3.9 – 1980 ppm).[13] Dusts with even higher concentrations of vitamin A might allow crickets to maintain adequate concentration of vitamin A for longer than 1 hour post-dusting. High vitamin A concentration may be toxic and unstable, raising concerns about dust handling, product effective shelf-life and safe dosing, however current dust retinol levels are considerably lower than levels shown to cause toxic effects in carnivores (24,738 ppm DM).[4]

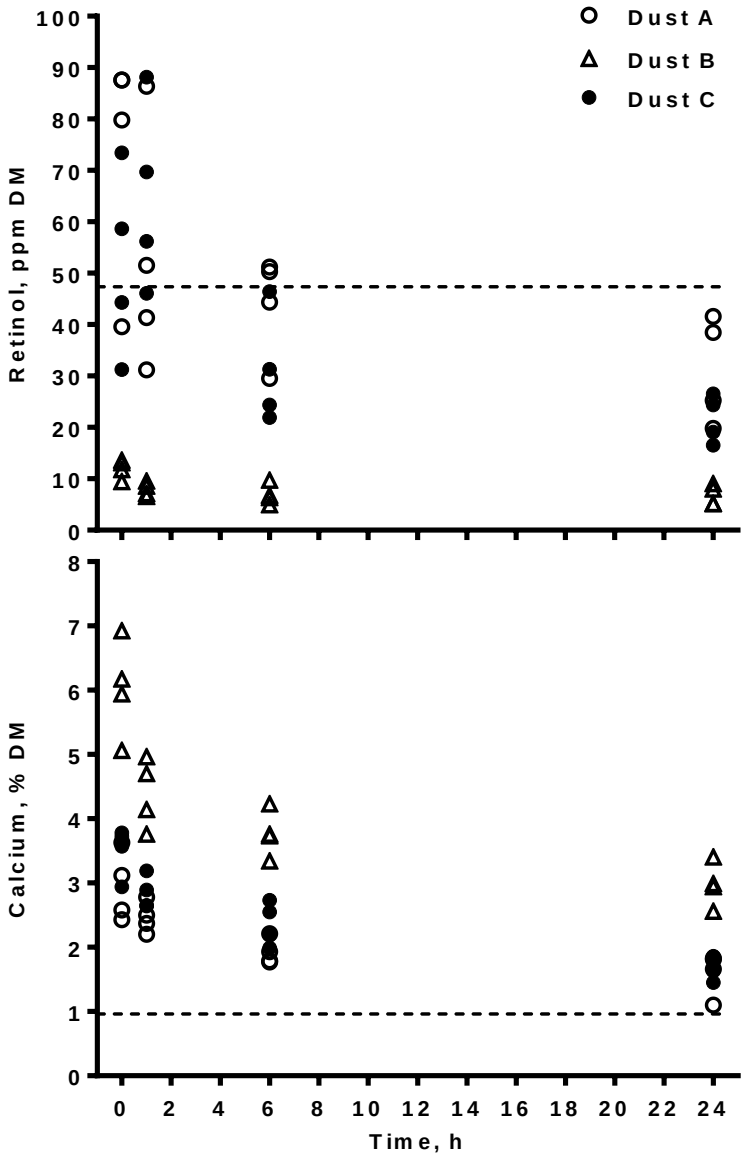

Figure 1: Retinol and calcium concentrations of crickets dusted with 50% calcium carbonate + 50% Nekton Rep vitamin/mineral supplement (Dust A), Repashy Vitamin A Plus (Dust C), or Repashy Calcium Plus (Dust B) after 0, 1, 6, or 24 h. Target concentrations for insectivores were set as 47 ppm retinol and 0.95% calcium (dotted lines).[2,5]

Dust B and C appeared to coat crickets evenly, but Dust A did not seem to stick to the cricket's body and instead formed clumps around the head or cerci. Clumping of dust did not appear to reduce the effectiveness of dusting. In fact, relative retinol adherence to crickets appeared to be greater for Dust A than Dust C as lower baseline retinol in Dust A resulted in similar cricket retinol concentrations. Adherence of dust is an important factor in determining the effectiveness of dusting - i.e. baseline concentrations of supplements alone does not guarantee effective transference of nutrients to the cricket and ultimately the cricket consumer.

These results suggest that alternative methods to dusting for supplementing vitamin A are required for animals that do not immediately consume their cricket diet - for example gut loading crickets with a vitamin A diet[5] and then dusting with calcium or use of a dust with higher vitamin A concentration. A stronger definition of target ranges for nutrients in insectivorous amphibians is needed to ensure appropriate nutrient levels are met.

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MILK COMPOSITION OF THE BONGO ANTELOPE (*TRAGELAPHUS EURYCERUS*) THROUGH LACTATION

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Introduction

Lactation is a fundamental adaptation of mammals, with milk being the first food for all mammals. The diversity of mammals is reflected in the diversity of lactation strategies and the composition of milk from different species. Although all milks have the same basic nutrients in common, the proportion of fat, sugar, protein and minerals varies widely among species (Oftedal and Iverson, 1995; Langer, 2008). This has implications for understanding the reproductive strategies of different species, the differences in patterns of growth for the young, and practical implications for zoological parks that are often faced with the necessity of hand rearing different mammalian species.

Bongo antelopes (*Tragelaphus eurycerus*) are a species of antelope that originate from Africa. The gestation length is about 285 days. Bongo antelope calves weigh about 20 kg when born. Adult females weigh between 210-230 kg, while adult males weigh 240-270 kg. Young reach sexual maturity when approximately 2 years old.

Methods

Milk samples were part of the Smithsonian National Zoological Park's Milk Repository and had been stored at -20°C since collection. For this study 23 Bongo (*Tragelaphus eurycerus*) samples from 2 individuals were analyzed. Milk samples were analyzed from day 6 post-partum through day 300 post-partum.

Samples were assayed for dry matter (DM), fat, sugar, crude protein (CP), ash, calcium, and phosphorus, and gross energy using standard methods that have been validated at the Nutrition Laboratory of the Smithsonian National Zoological Park and performed on milks from about 200 species of mammals. Briefly, for DM, milk samples were aliquoted, weighed, and dried in a forced air convection drying oven for 3.5 hours at 100ºC and then reweighed (AOAC, 1990). Total nitrogen (TN) was determined for the dried milk samples using a carbon, hydrogen, and nitrogen (CHN) elemental gas analyzer (Model 2400, Perkin Elmer, Norwalk, CT). This method has been validated against the macro Kjeldahl procedure with nitrogen recovery around 98-99%, and has been used at Smithsonian National Zoological Park to measure milk nitrogen for a wide variety of species. The obtained nitrogen value was multiplied by 6.38 to determine the amount of CP in the milk. Total lipid was measured using a micro modification of the Roese – Gottlieb procedure by means of sequential extractions with ethyl alchohol, diethyl ether, and petroleum ether. Total sugar was analyzed by the phenol – sulphuric acid colorimetric procedure (Dubois et al., 1956; Marier & Boulet, 1959) using ultraviolet spectroscopy and lactose monohydrate standards. Ash was determined by placing dried milk samples in a muffle furnace at 550°C for 8 hours. Gross energy was calculated using the formula: 9.11 * fat + 3.95 * sugar + 5.86 * CP (Perrin 1958). Calcium was measured using atomic absorption spectrophotometry. Phosphorus was determined by a colorimetric procedure and read with a microplate reader at 405nm.

Values are expressed on a wet weight (As-Is) basis, both as g/g (%) and on a per energy basis (mg/kcal).
The mg of nutrient per kcal of milk was calculated by: 1000 * (nutrient expressed in g/g)/GE.

Results and Discussion
Bongo antelope milk is quite high in dry matter (28.2%) (Table 1). The milk is additionally higher in fat than sugar (11.8% versus 3.5%). Most of the energy in the milk is provided from fat. Additionally, the protein content of Bongo antelope milk is very high, about 60 mg protein per kcal. In comparison, cow's milk contains approximately 48 mg protein per kcal. The high protein levels of Bongo antelope milk suggest a faster growth rate for fawns. The ash content of Bongo antelope milk is similar to that of cow's milk at around 1.0%. The high ash content is also consistent with a faster growth rate. The calcium:phosphorus ratio of 2.2 was expected in order to support healthy bone growth.

There was very little change in the percentage of each nutrient over the lactation period with the exception of the last two samples (days 286 and 300 post-partum). It is likely that the fawn stopped suckling prior to day 286 post-partum. There was also very little change over lactation when looking at crude protein, sugar, and fat on a mg per kcal energy basis (Figure 1). It is of interest that, unlike many mammals, only very minimal changes were seen in the milk composition over the lactation of the Bongo antelope. Some changes are likely to occur between colostrum and later milk samples; however, no colostrum samples were analyzed in this study. Additional changes in milk composition, especially in energy, occur as growth rate changes. For example, in the White rhinoceros, milk energy decreases after 3 months of lactation. However, no similar changes were observed over the lactation of the bongo antelope. This suggests the growth rate of Bongo antelope fawns does not decrease as the fawn gets older.

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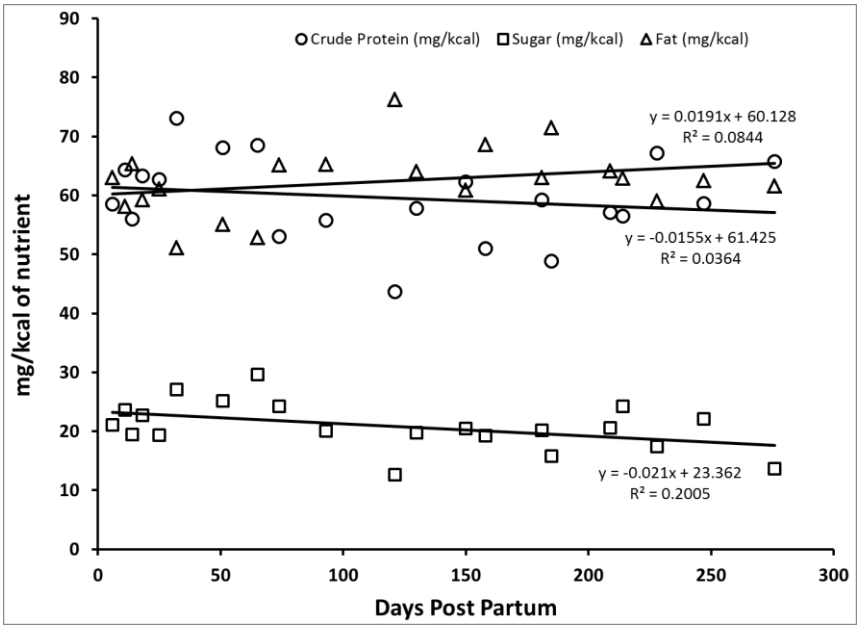
Table 1. Nutrient composition of Bongo antelope milk expressed as a percent of total milk.

Nutrient	Amount (% ± SE)
Dry Matter	28.2 ± 1.1
Crude Protein	10.9 ± 0.3
Sugar	3.5 ± 0.2
Fat	11.8 ± 0.8
Ash	1.0 ± 0.05
Calcium	0.29 ± 0.01
Phosphorus	0.13 ± 0.01
Ca:P Ratio	2.17 ± 0.1

Figure 1. Changes in crude protein, sugar, and fat on a gross energy basis over lactation in bongo antelopes. The last two samples were not included due to significant alterations in the milk nutrient composition compared to the other samples.



GROWTH FACTORS AND METABOLIC HORMONES IN PRIMATE MILK.

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Introduction

Milk is more than just a food. It is also a means by which mammalian mothers signal biochemically to their offspring. Milk contains physiological levels of many growth factors, cytokines, and hormones. A broad spectrum of evidence suggests that these signaling molecules have important effects on growth and development of neonates. For example, feeding preterm infants human breast milk dramatically reduces their risk of developing necrotizing enterocolitis, a major source of morbidity in preterm babies. The specific signaling molecules in breast milk that act on the infant's intestinal tract are not known, although epidermal growth factor (EGF) and transforming growth factor β-2 (TGF-β-2) are logical candidates. Both of these growth factors are found in breast milk, and both have significant effects on intestinal epithelial cell proliferation and maturation (Coursodon and Dvorak, 2012). Many other metabolic hormones found in milk (e.g. leptin, adiponectin) have been suggested to play a role in the development of infant metabolism, and to be potential risk factors for early onset of obesity and type-2 diabetes (Savino et al., 2009; 2011). There are also many immune function molecules (e.g. secretory IgA) and inflammatory cytokines (e.g. IL-1β, IL-6, TNF-α) in milk. The infant's immune system is known to be immature, and to rely on these immunological signals from the mother via milk. It would appear that other organ systems related to infant growth, development and metabolism are also not fully functional, and are strongly affected by signaling via milk as well.

Validation of animal models to examine the functional significance of milk-borne signaling molecules on infant health and development would be of great benefit. The purpose of the pilot study reported here was to investigate whether assays designed to measure two growth factors (EGF and TGF-β-2), receptors for these two growth factors, and the metabolic hormone adiponectin in human milk would also work in non-human primate milks. In addition, the pattern of expression of these molecules in milk was examined in milk samples from a gorilla obtained from the birth of her infant and then every week through 40 months of age.

Methods

Milk samples for gorilla, orang utan, baboon and common marmoset came from the Smithsonian National Zoological Park's Milk Repository; milk samples for rhesus macaque came from the California National Primate Research Center's milk collection, courtesy of Dr. Katherine Hinde. The milk samples from baboon (n=9) and common marmoset (n=5) represented a single time point in mid lactation from multiple females. These samples were pooled within species. The rhesus macaque samples were also from multiple females (n=20) and represented two time points: early lactation and mid lactation. The orang utan milks were from a single female from

infant age 6 months through one year. The gorilla samples were from a single female and were collected every week from the first week of life until the infant was 40 months old.

EIA kits

Parallelism and recovery protocols

We performed parallelism and recovery tests to validate that the component kits were able to accurately measure our analytes of interest. Briefly, we pooled several aliquots of milk from different time points, or from different individuals (where available), for each species. These pooled samples were serially diluted and run in an assay along with the standard curve supplied by the manufacturer. We then tested the parallelism of the slopes of the standard and serially diluted pooled curves. Since the component kits used were not developed for use with milk, we utilized recovery tests in order to test whether anything in the sample matrix interfered with the assay. To do this, we spiked samples from our species of interest with controls provided in the kit, and measured the % value recovered.

Adiponectin, We measured adiponectin using the human Adiponectin DuoSet ELISA Development kit, (R&D Systems, Inc., Minneapolis, MN), following the manufacturer's protocol. Gorilla and orangutan milks were run at a dilution of 1:3, and baboons at 1:15.

EGF. We measured EGF using the human EGF DuoSet ELISA Development kit (R&D Systems, Inc., Minneapolis, MN), following the manufacturer's protocol. Gorilla samples were run at 1:300, orangutan samples at 1:80, and baboons at 1:25.

EGF-R. We measured EGF-R using the human EGF R DuoSet ELISA Development kit (R&D Systems, Inc., Minneapolis, MN). We implemented alterations of the manufacturer's protocol in the following ways: the capture antibody was used at 0.5 ug/ml, the detection antibody used at 150 ng/ml, the reagent diluent included 0.05% Tween 20 in addition to the 1% BSA, and the washing steps included a 30-second soak. Gorilla samples were run at 1:10, and orangutan samples were run at 1:5.

TGFbeta2. We measured TGFB-2 using the Quantikine Human TGF-β2 Immunoassay kit (R&D Systems, Inc., Minneapolis, Mn), following the manufacturer's protocol. Individual milks validated were not diluted before or after activation, which yields a dilution factor of 7.8.

TGFbetaR3. We measured TGFBR-III using the human TGF-β RIII DuoSet ELISA Development kit (R&D Systems, Inc., Minneapolis, MN), utilizing the following alterations to the manufacturer's protocols: the detection antibody was used at 100 ng/ml, and the washing steps included a 30-second soak. Gorilla samples were run at 1:40, orangutan samples at 1:20.

Results

All analytes were successfully measured in gorilla milk. Concentrations of TGF-β-2 were below detection in orangutan milk, and those of TGF-β-R3 were below detection in macaque milk. EGF and adiponenctin were successfully detected in a pooled baboon sample. Tests for the other analytes are ongoing. Concentrations of EGF and adiponectin were below detection in marmoset milk; there was not sufficient sample to test the other analytes. All other parallelisms for

measurable analytes indicated in Table 1 showed a correlation between slopes of serially diluted pooled samples and the standard curve of R2>/=0.98. All recoveries were >/= 96%.

There were species differences in mean concentration and range of all analytes (Table 1). In gorilla milk there was a pattern of high analyte concentration in early infancy declining to a lower, steady-state concentration until about three years of age, when several analytes displayed an increase in concentration (Figures 1 and 2). The parallel increase in EGF and its receptor at 3 years is intriguing (Figure 2) suggesting that the receptor might be acting as a binding protein in this context. It is not certain how much milk the infant is consuming at this age, though the female continues to lactate and provide sizeable milk samples.

Discussion

The assay kits were designed to measure these analytes in human samples. All the assays were successful in gorilla milk, the phylogenetically closest species to human in this study. In the species furthest from humans (common marmoset) neither of the two assays attempted detected any analyte. In Old World monkeys and an Asian great ape, four of five assays were successful; however, the assays that failed to detect any analyte differed between orang utan and rhesus macaque. We cannot tell from these data whether the assays for TGF-β-2 and TGF-β-R3 failed in orang utan and rhesus macaque, respectively, or that these molecules are not excreted into their milks at any appreciable level.

The longitudinal data from gorilla suggests that colostrum and early milk contain greater concentrations of bioactive molecules than does mature milk. However, the levels in mature milk of these analytes are significant, and they could have important function when ingested by infants. Maternal biochemical signaling would appear to continue throughout lactation. The significance of the increases of adiponectin, EGF and its receptor at 3 years is not known.

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Table 1. Assay results for primate milks.

	EGF	EGF-R	TGF-β-2	TGF-β-R3	Adiponectin
Gorilla	N=95	N=95	N=78	N=79	N=95
Mean	29,663.72	2,063.15	6,817.40	111,596.23	4,812.59
Range	9,446.70-68,078.70	626.57-13,806.64	261.77-69,369.85	56,176.56-512,590.29	1,309.27-34,693.64
SEM	929.821	218.08	1,232.87	6,089.46	476.82
Orang utan	N=16	N=16	Not detected	N=16	N=16
Mean	7,764.45	598.87		24,741.05	1,399.45
Range	3,469.44-9,756.56	254.57-740.41		8,199.46-36,873.74	562.70-1,919.24
SEM	371.70	29.37		1,866.65	87.32
Rhesus macaque	N=40	N=40	N=33	Not detected	N=40
Mean	2,877.83	409.66	414.62		6,918.96
Range	860.05-6,660.90	126.97-1,140.26	91.78-1,372.08		2,340.31-18,707.13
SEM	206.52	34.53	58.18		441.12

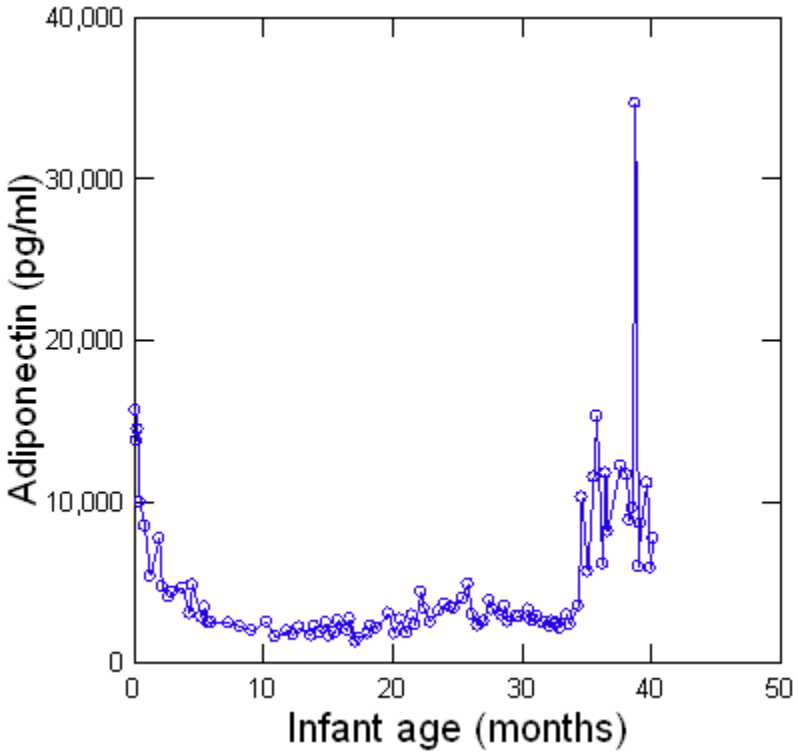

Figure 1. Concentration of adiponectin in gorilla milk as a function of infant age.

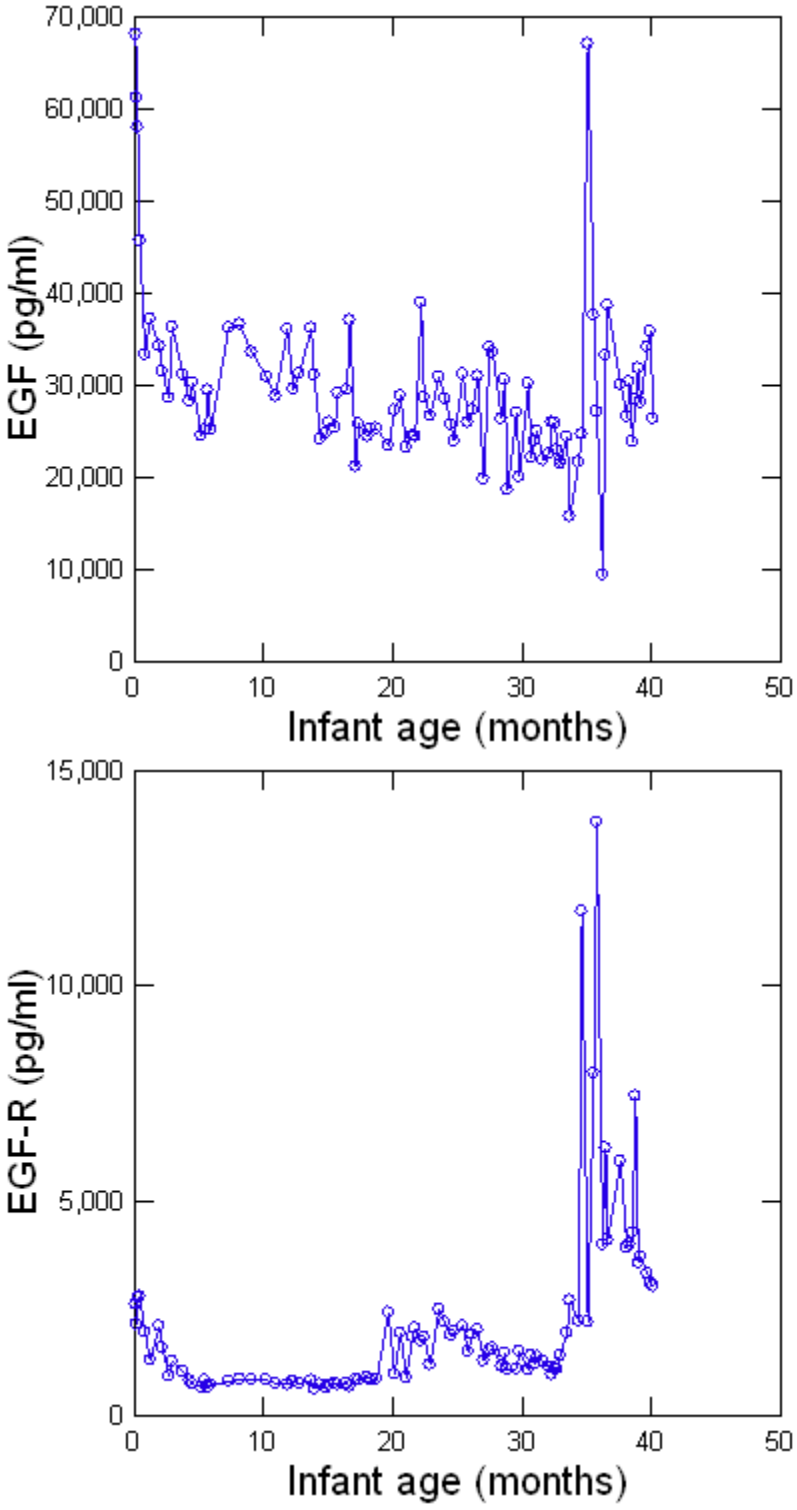

Figure 2. Concentration of EGF and its receptor in gorilla milk as a function of infant age.

TESTING CAROTENOID CONVERSION IN BULLFROGS (RANA CATESBEIANA) AT MULTIPLE LIFESTAGES

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To maintain amphibians healthily in captivity it is important to determine the appropriate type of vitamin A for the different life stages of these species. It is not known if there are differentiating needs between pre-formed vitamin A and vitamin A precursors (such as carotenoids) for proper supplementation of pre-metamorphosized (tadpole) or full adult stages in captivity. These animals progress from a diet of primarily vegetarian items as tadpoles to one predominantly of insects and other similar prey items upon reaching maturity (fully metamorphosized) (Pond and Bell, 2005). Because carotenoids are generally considered to be a "safe" nutrient for vitamin A supplementation (unlike the potentially toxic pre-formed vitamin A), it is important to determine more precise nutrient utilization by amphibians to ensure marketed complete feeds contain the correct species specific vitamin A nutrient. Preliminary results evaluating the conversion of beta-carotene to vitamin A by adult frogs and toads found the conversion to be very minimal (McComb, 2011).

The objective of the current research was to determine the presence of biological pathways for conversion of carotenoids in the organs of tadpole and adult bullfrogs (*Rana catesbeiana*). Liver and small intestine from captive animal were processed to achieve an enzyme fraction. These tissues were utilized because carotenoids are absorbed into the intestinal cells where some of them can be converted to vitamin A. The rest of the carotenoids are absorbed intact and are delivered to the liver for storage and possible conversion at a later time. For this reason, the presence of vitamin-A producing enzymes is most noted in the small intestine and liver, for species that have the enzymes (Erdman et al, 1993). This primary goal of this research was to evaluate the enzyme presence and resulting potential vitamin A conversion within the small intestine and liver.

Frog small intestine and liver samples were incubated with one of three carotenoid treatments containing beta-carotene, beta-cryptoxanthin, or canthaxanthin. High performance liquid chromatography (HPLC) will be used to quantify the vitamin A of the incubated samples and the net endogenous vitamin A produced will be calculated for each treatment. Due to the novelty of this research technique, correct HPLC methods have been difficult to ascertain and these challenges will be further documented.

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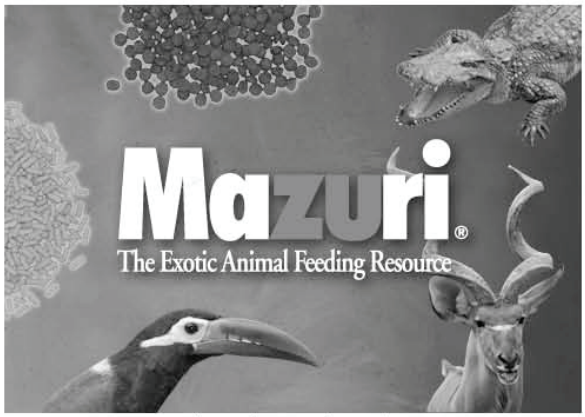

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