First Annual Crissey Zoological Nutrition Symposium

Raleigh, North Carolina

December 12 & 13, 2003
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The Crissey Zoological Nutrition Symposium is an Environmental Medicine Consortium Project supported by the generous contributions of donors to the Environmental Medicine Endowment of the North Carolina Veterinary Medical Foundation.
First Annual Crissey Zoological Nutrition Symposium  
Raleigh, North Carolina  
December 12 and 13, 2003  

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, micronesia

kingfishers, and many more, to illustrate her talks and discussions. Sue published over 100 scientific papers including several seminal topical reviews. In 2002 she was awarded the Duane E. Ullrey Achievement Award by the American Association of Zoo Veterinarians for her distinguished work.

Sue loved her North Carolina farm, and maintained a significant menagerie of zoo retirees and castaways there, commuting from her home in Burgaw, to Chicago to manage her zoo duties, and traveling to Raleigh at the drop of a hat to teach. Sue was a meticulous scientist whose enthusiastic joys of teaching and insistence on “good science” have become part of those who were lucky enough to be around her for any length of time. Future generations of zoological nutritionists are richer for 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
Dr. Jay Kaplan (B.A. Swarthmore College, M.A. and Ph.D., Northwestern University), is a Professor of Comparative Medicine and Associate Director of the Comparative Medicine Clinical Research Center at the Bowman Gray School of Medicine, Wake Forest University. A physical anthropologist and primatologist, Dr. Kaplan investigates the influence of psychosocial stress on disease. His latest research major research interest is to understand the role played by social behavior in the development of atherosclerotic heart disease among males and premenopausal females. Recently, his team has begun to explore the neurobiologic concomitants of behaviors that are associated with disease susceptibility and resistance. This research led to the unanticipated finding that alterations in dietary cholesterol are associated with changes in central serotonergic activity as well as social behavior. Dr. Kaplan was recently featured on the PBS series Scientific American Frontiers: Worried Sick with host Alan Alda. The show’s segment “Angry at Heart”.

Recent Publications


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A COMPARISON OF FIBER USAGE ACROSS FOUR LEMUR SPECIES THAT VARY IN DIETARY PROFILE: VARECIA VARIEGATA, EULEMUR FULVUS, PROPITHECUS VERREAUXI, AND HAPALEMUR GRISEUS

Jennifer L. Campbell Ph.D.1*, Cathy V. Williams D.V.M.2, and Joan H. Eisemann Ph.D.3

1Department of Zoology, North Carolina State University, Raleigh NC 27695; 2Duke University Primate Center, Durham NC 27705; 3Department of Animal Science, North Carolina State University, Raleigh NC 27695.
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Free-ranging lemur species consume diets that vary in both amount and type of fiber. Total dietary fiber (TDF) analysis quantifies both the soluble fiber (SF) and insoluble fiber (IF) portions of a foodstuff1,2, and feeding ecology studies suggest that two species, Varecia variegata (Vv) and Eulemur fulvus (Ef) consume a frugivorous to a frugivorous-folivorous diet with more SF relative to Propithecus verreauxi (Pv) and Hapalemur griseus (Hg), folivorous species that consume more IF by comparison.3 These diet differences have likely resulted in differences in digestive efficiency. Two diets that were similar in TDF content (28% of dry matter) yet possessed different ratios of IF:SF were formulated to test differences in digestibility across these four species. The high IF diet (InsF) possessed a ratio of 12.15:1 and the mixed fiber diet (MixF) possessed a ratio of 3.76:1. The dry matter digestibility (DMD) of MixF (67.3%) was higher than InsF (63.8%) (p<0.05, SEM 0.9%) across all species. The DMD of both diets differed across species: 55.5% for Vv, 58.6% for Ef, 71.8% for Pv, and 76.3% for Hg (p<0.05, SEM 1.4%). The TDF digestibility was 22.6% for Vv, 28.3% for Ef, 55.9% for Pv, and 66.1% for Hg (p<0.05, SEM 3.3%). The IF digestibility was 19.7% for Vv, 27.6% for Ef, 53.0% for Pv, and 62.3% for Hg (p<0.05, SEM 3.4%). Results for SF digestibility showed the same trend as IF. Results for Ef and Vv were generally similar and much lower than results for Pv and Hg. These data complement information obtained from feeding ecology studies and are useful in planning the nutritional management of captive lemurs.


Traditional methods of studying nutrition in wild animals have historically consisted of observational, physical, and chemical analysis of ingested food items and macroscopic analysis of fecal samples or intestinal tract contents. The current revolution in molecular biology offers new methodologies for nutritional analysis that are only beginning to be explored. This presentation will briefly discuss the theoretical issues relating to using this technology for investigation of wild mountain gorilla nutrition. Particular attention will be paid to the potential strengths and weaknesses of using polymerase chain reaction (PCR) on DNA extracted from fecal samples.
The purpose of this work was to measure important nutritional concentrations for a group of captive Guinea baboons (Papio hamdyrias papio) and compare these data to published values for primates. The nutritional concentrations of 55 captive guinea baboons were examined and blood samples were analyzed for serum chemistry (alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, blood urea nitrogen (BUN), chloride, cholecystokinin, CO₂, creatinine, gamma-glutamyltransferase, glucose, total protein and uric acid), carotenoids (α and β-carotene, lutein/zeaxanthin, lycopene, α- and β-cryptoxanthin), lipids (high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, total cholesterol and triacylglycerides), and vitamin D metabolites 25(OH)D (25-hydroxycholecalciferol) and 1,25(OH)₂D (1,25-dihydroxycholecalciferol), A (retinol and retinyl palmitate) and vitamin E (α and γ-tocopherol). Each concentration was statistically evaluated by both sex and age group (5-9 yr, 10-15 yr and > 15 yr). Alanine aminotransferase, calcium, chloride and total cholesterol were higher for females than males and phosphorous was higher for males than females (P<0.05). Alpha - tocopherol, cholecystokinin, retinol and triacylglycerides tended to be higher for females than males (P<0.10). Total bilirubin decreased as age increased (P<0.05) and alanine aminotransferase tended to decrease as age increased (P<0.10). Alkaline phosphatase and retinol were lower for the middle age group compared to the youngest age group (P<0.05). Alpha - carotene, calcium and retinyl palmitate increased with age (P<0.05) and β - carotene and low density lipoprotein cholesterol tended to increase with age (P<0.10). Although individual diet intake was not measured, the composition of the diet offered to the baboons was known and nutritional concentrations were compared to diet. The diet met the current standards for baboons. With the exception of 25(OH)D, all serum measurements were within the ranges of the published values for cercopithecidae, great apes and humans and therefore did not indicate abnormalities. The mean for 25(OH)D (88.2 ± ng/dl) was higher than published data for cercopithecidae, great apes and humans (13 - 55 ng/dl).¹ ² ³ This information provides a large collection of new data from a healthy troop of Guinea baboons for comparison to other captive primates and free-ranging primates in general.
References:


HOW MILK COMPOSITION DOES, AND DOES NOT, VARY WITH MATERNAL CONDITION IN THE COMMON MARMOSET (CALLITHRIX JACCHUS).

Michael L. Power Ph.D., Olav T. Oftedal Ph.D., Suzette D. Tardif Ph.D.

Smithsonian's National Zoological Park, Department of Conservation Biology, Washington DC 20008
Southwest National Primate Research Center, P.O. Box 760549, San Antonio TX 78245-0549

The composition of milk depends upon many factors; for example, phylogeny, nutritional status of the female, and stage of lactation period. We present data on the composition of milk of the common marmoset (Callithrix jacchus) and examine variation in milk composition across lactation and among females that differ on a measure of nutritional status (weight change during lactation).

Methods
Females were separated from their infants for at least three hours to allow milk to collect in the mammary glands. Females were anesthetized using ketamine and injected with oxytocin (2 IU intramuscularly). Milk was manually expressed from each teat, and collected into 1.8 ml cryovials. Milk samples were stored frozen at -20°C until they were assayed.

Samples were collected at approximately 20, 32, and 45 days post partum during each lactation period. A few samples were collected at the end of lactation, between days 65 and 75 post partum. Most females had milk samples from only one or two different litters, but four females had samples from three different litters, one female from four different litters, and another female from five different litters. Milk samples were assayed for dry matter (DM), nitrogen, fat, sugar, and calcium (Ca) at the Nutrition Laboratory of the National Zoological Park. Crude protein (CP) was calculated by 6.38 X nitrogen, and gross energy (GE) was calculated using 5.86 kcal/g CP, 9.11 kcal/g fat, and 3.95 kcal/g sugar.

Females were weighed weekly starting within days of parturition. Weights through day 70 post partum were analyzed by linear regression. By day 70, lactation is essentially completed for most females, and there will have been little mass gain of placenta or fetuses for those females that became pregnant in the first weeks post partum. Females were classified as having lost weight, maintained weight, or gained weight for each lactation period. Multivariate analyses of covariance was used to examine the variation among DM, CP, fat, sugar, and GE using weight-change classification as the categorical variable and maternal age and infant age as the covariates.

Results
A total of 110 milk samples were collected from 25 adult female common marmosets. Not all samples were of sufficient volume for complete proximate analysis (DM, CP, fat, and sugar). A total of 78 milk samples from 22 females had complete assay results. Among those 78 samples, 61 samples were also assayed for calcium. Mean values for these constituents are presented in Table 1.
Table 1. Mean values for the constituents of milk of common marmosets. Values are mean (SD). (N = number of females, n = number of samples, DM = dry matter, CP = crude protein, GE = gross energy).

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<th>N</th>
<th>n</th>
<th>DM (%)</th>
<th>CP (%)</th>
<th>Fat (%)</th>
<th>Sugar (%)</th>
<th>GE (kcal/g)</th>
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<td>22</td>
<td>78</td>
<td>14.4 (2.7)</td>
<td>2.8 (0.7)</td>
<td>3.5 (1.9)</td>
<td>7.3 (0.7)</td>
<td>0.76 (0.18)</td>
<td>0.11 (0.04)</td>
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Variation across lactation appeared to be random; there was no consistent change in milk composition with infant age. There was considerable variation in the concentration of milk constituents among the females in this data set. Even among litters for the same female there was considerable variation in milk composition.

Milk samples from lactation periods where a female gained weight (n = 23) were higher in DM, CP, fat, GE (p = .001 for all) and Ca (p = .019), but not different for sugar (p = .686) compared to milk samples where a female either maintained weight (n = 27) or lost weight (n = 28). Neither maternal age nor infant age was a significant factor affecting milk composition.

If CP, fat and sugar were expressed as their percent of estimated GE, females that gained weight had a higher percent of GE from fat (p = .047). However, weight change was not a significant factor for the percent of GE from CP (p = .851) and females that gained weight had a lower percent of GE from sugar (p = .009). Neither maternal age nor infant age was a significant factor. When the Ca concentration of milk samples was analyzed by weight change with GE as a covariate, then GE was a significantly positively associated with Ca (p = .010), but weight change was no longer significant (p = .733)

Discussion

In the common marmoset, female condition affects some, but not all properties of their milk. Females that gained weight during lactation produced milks that were higher in DM and GE, and had a higher proportion of energy from fat and a lower proportion of energy from sugar. The concentrations of both CP and Ca appear to be consistent proportions of GE regardless of female weight change.

The proportion of GE from CP may be a trait that displays phylogenetic variation within anthropoid primates. The value is low for humans and great apes (about 6%), moderate for Old World monkeys (about 12%), and high for New World monkeys (about 20%). Among prosimians, it is moderate for *eulemur* spp. (about 13%) and high for lorises (about 21%). The proportion of GE from CP may also be related to the different growth rates among these groups, as growth rate adjusted for maternal metabolic size follows the same pattern, with humans and great apes having the lowest adjusted growth rates and New World monkeys and lorises having the highest.
A RETROSPECTIVE ANALYSIS OF IRON DEPOSITION IN A LARGE COLONY OF CAPTIVE LEMURS

Kelly M. Glenn1; Jennifer L. Campbell Ph.D.2*; and Cathy V. Williams DVM1

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Hemosiderosis, also called iron storage disease, is widely considered to be a significant health concern for institutions housing captive prosimians.1-3 Published reports suggest that lemurs are more susceptible to excessive iron accumulation than other species, and that diet modifications intended to decrease iron absorption are recommended as a preventative measure.3, 4 Excessive accumulation of hemosiderin, an iron storage protein, in the liver as well as other body tissues is an indicator of the disease in other species.5 In order to assess the extent of hemosiderosis in a captive lemur colony, histopathology reports, iron-specific staining (Prussian Blue6) of liver tissue, and quantitative analysis of liver iron were used to determine iron deposition across 15 lemur species. All animals were housed at the Duke University Primate Center (Durham, NC) and tissues were harvested during a routine necropsy procedure. All animals that died between 1990-2002 and were over 6 months of age were included in the study. The presence of hemosiderin pigment was mentioned in 31% (n=153) of the histopathology reports, and descriptions varied from incidental to severe. The mean total iron score (TIS), hepatic iron score (HIS), and sinusoidal iron score (SIS) was 16.7 ± 1.8, 11.6 ± 1.4, and 3.69 ± 0.5, respectively (n=49). Across all species, scores increased with age (p<0.05). The mean liver iron concentration (wet weight) for all species was 1170.2 ± 117.03 µg/g (mean ± SE; n=129), and ranged from 214.5 ± 30.61 µg/g in the diurnal Lemur catta to 4334.5 ± 1099.2 µg/g in Cheirogaleus medius, a small nocturnal lemur. Results indicate a wide range of iron deposition across the 15 lemur species tested.

References:
A PRELIMINARY EVALUATION OF NON-INVASIVE METHODS TO SCREEN FOR IRON OVERLOAD IN LEMURS

Cathy Williams DVM*1, Jenny Campbell Ph.D2, and Kelly Glenn1

1Duke University Primate Center, Durham, NC 27705; 2Department of Zoology, North Carolina State University, Raleigh, NC 27695. e-mail: cathy.williams@duke.edu

A syndrome of excessive iron accumulation (hemosiderosis) was first recognized in captive lemurs in the 1960’s and descriptive reports of the condition were published in the 1980’s.1-3 All reports describing iron overload in lemurs have been done on post-mortem specimens. Given that excessive tissue iron deposition is non-reversible once it occurs, it is important to explore ways to evaluate the iron status of living lemurs if preventative measures are to be instituted early enough to alter the course of the disease. Currently, it is necessary to perform a liver biopsy to obtain an ante-mortem diagnosis of iron storage disease in lemurs. In humans and some domestic species it is possible to screen for iron overload noninvasively by measuring serum levels of total iron (SI), total iron binding capacity (TIBC), and ferritin. The percent transferrin saturation (%TS) is then calculated as SI/TIBC.4,5 Normal values for these tests are not available for lemurs. A prospective study was undertaken to determine iron analyte values in 203 healthy lemurs comprising 11 species at the Duke University Primate Center. The mean, standard error, and range of values for SI, TIBC, ferritin, and %TS were calculated for each species. Pearson correlation coefficients were calculated to determine if relationships existed between tests without regard to species.

Mean values for SI in the different species ranged from 102.5 - 278.8 μg/dL, from 349.7-533.6 μg/dL for TIBC, 32.8-222.1 ng/mL for ferritin, and 27.9-53.6% for %TS. There were significant differences (P<.05) in the mean values for the different tests between several species. Significant positive correlations existed between increasing age and ferritin (P=.0002) and increasing iron and TIBC (P<.0001). A negative correlation was found between ferritin levels and TIBC (P=.0165). Additional tests are necessary to determine how closely serum iron analyte values correspond to total body iron stores in lemurs.

References
RETURN TO HEALTH: TOWARD MORE NATURAL DIETS

Astrid MacLeod 1,2
Janine Perlman 1,2
Lee Theisen-Watt 1*
Michele Martino, co-presenter 1,3*

1 Advanced Primate Ethical Studies
2 Wildlife Nutrition Consultants
3 Dept. Veterinary Sciences, M.D. Anderson Cancer Center

Introduction
Optimizing the health and life span of captive primates are goals of every zoo and primate facility. How can we achieve these goals? Some species seem to thrive; many others, however, suffer morbidity and premature mortality. Often, captive primates’ diets vary greatly from their natural diets. These two facts are not coincidence; few would dispute that, to a large extent, they are cause and effect. One example all too familiar to zoo staff is “Wasting Marmoset Syndrome” in callitrichids (Ialeggio and Baker, 1995), but numerous primate species are considered difficult to maintain in healthy condition.

In this paper we present two “anecdotes” in which we used fundamental principles of digestive physiology and nutritional analysis to bring captive primates from physical decline back to health, using an array of foods that matched as closely as possible the animals’ natural diets. While these observations do not qualify as case studies, they provide a basis for further investigation.

Background
Although primate species occupy a wide array of trophic niches, most primates are mainly frugivorous and/or folivorous.

In the wild, each species consumes a particular ratio of various plant parts that include young, tender leaves, mature leaves, fruits; buds, flowers, sap, nectar, and less commonly, roots, tubers, stems, fungi, bark, etc. Animal foods, generally invertebrates, are often a small but presumably important source of protein and certain micronutrients (e.g., some minerals and fatty acids).

The ratio of plant parts may vary seasonally (e.g., young vs. mature leaves; flowers vs. fruits), and the most nutritious foods available are generally selected. Typically, while a dozen or fewer plant species may be eaten in a day, primates consume in excess of 125 plants species over the course of a year (Milton, 1999). Selections of plant species and parts of plants appear to be meaningful; for example, some primates carefully choose particular portions of leaves. Purposeful selection of plant parts is postulated to relate to avoidance and/or requirement of specific phytochemicals that may be crucial at particular times of the plant’s or animal’s life. (E. Munoz, pers. communication; Robbins, 1995).
Wild and cultivated plants differ in numerous ways. Wild plants often contain higher concentrations of micronutrients. Wild fruits are typically lower in total sugars and sucrose, and higher in monosaccharides and fiber (Milton, 1999). Notably absent from natural diets are grains and legumes.

Most folivorous/frugivorous primates rely on fermentation to digest the large amounts of fiber they require. They typically possess a large, sacculated colon, although colobines are foregut fermenters with complex, chambered stomachs. Enormous populations of specific gut microbes digest and ferment a wide variety of carbohydrates from the natural diet, thus supplying a significant portion of energy requirements. They also serve to competitively exclude pathogens, and to detoxify secondary plant compounds.

The primate’s eating behavior and nutritional requirements, the plants that comprise its diet (with their very specific carbohydrates), and the animal’s gut function and symbionts, have coevolved over millions of years. The resultant multidirectional mutuality is exquisite, delicate, and essential to the animal’s health. A number of aspects of digestive physiology and nutritional requirements in relation to captive feeding of folivorous primates are discussed by Nijboer et al. (1997).

**Methods**

In 2003, we had occasion to consult on the diets of several captive primates whose physical condition was in decline. In each case, the animals were on diets typical of facilities that provide enriched environments and somewhat varied diets. Those diets did not incorporate the above biological realities, with their consequent mandates. Compared to the animals’ requirements, the diets were very high in grain- and/or tuber-based carbohydrates and a limited variety of often sucrose-rich fruits, while the variety and amounts of leafy foods were very low.

In one case, an adolescent male siamang (Hylobates syndactylus) was transferred from a facility that provided a varied and nutritious diet, to a facility that typically fed the following meal BID: Boiled yellow potato, boiled sweet potato, 1 piece broccoli, 1 piece green onion, apple, banana, 1 leaf kale, 2 leaves romaine, 1 piece carrot, fresh tomato, 4-5 peanuts, and a few monkey biscuits. Applesauce and nonfat fruit yogurt were offered once between these two feedings. Processed human foods such as cake and hotdogs were fed irregularly, as a minority portion of the diet. Of all the offerings, bananas were eaten with the most consistency; other items were often left unfinished.

Over six weeks, the animal’s weight dropped from 7.9 kg to 6.6 kg. His muscle tone was poor; his thigh circumference decreased from 20 cm to 15 cm. He was lethargic; his skin was dry and cracked, with bleeding on digits. His coat was dull, with hair loss lateral to the ischial callosities; and there were ischial bony protrusions. Recurring infections necessitated antibiotic therapy.

We instituted significant dietary changes. Processed human foods were eliminated. The amounts and variety of green leafy cultivated vegetables and browse (e.g., ficus, mulberry, bamboo) were dramatically increased, to provide about one dozen types per
meal, and to comprise approximately half of the food offered (by weight). A variety of lower-sucrose fruits were added. Chicken, whole eggs (including shell) and a variety of insects, along with nuts, fish oil, and a limited amount of canine kibble, were offered as rich sources of protein, fats and accompanying micronutrients. Oystershell grit and pieces of a multimineral block were offered ad lib, and eaten with enthusiasm. We also fed fresh feces from a healthy conspecific, one week after both had begun the improved diet, and again three days later. This was an attempt to replace microflora lost through antibiotic therapy, and to inoculate with microbial populations better suited to a more natural diet (see, e.g., Persky and Brandt, 2000).

Primate biscuits, which are essentially micronutrient-fortified soy and grain, were removed from the diet. As mentioned, no primate eats significant amounts of grain or legumes in the wild. Because of the negative prebiotic/fermentative properties of starch, increasing reports of gliadin allergies, and the clear unsuitability of soy (including its non-natural carbohydrates, phytoestrogens, and outright toxicity in some species), we consider primate biscuits to be singularly poor choices for inclusion in primate diets.

We also consulted on a pair of male mantled howler monkeys (Alouatta palliata) that had recently been relocated. They were housed in an outlying enclosure at the facility, and had just been traumatized by vandal activity. Their testicles retracted, and they developed severe diarrhea and anorexia. Their diet had consisted of high fiber primate biscuits and some typical fruit and vegetable offerings. At the time of the consultation, they were eating only green bell pepper.

We once again advised inoculation with feces from healthy congenerics and a diet comprising a wide variety of browse and leafy produce, as well as fruits. Over the next few days, they avidly ate “mixed organic baby greens”, oranges, spinach, and rose buds.

Results

Within one week on the improved diet, the siamang’s muscle tone had been observably restored. Twenty-five days later, his weight had returned to 7.9 kg, and continued to increase thereafter. His skin, coat, vigor, and demeanor were greatly improved.

A weakness of this account is that, due to constraints at the facility, it was impossible to weigh the amounts of various foods consumed, either before the intervention or afterward. However, careful observations showed that the animal consistently ate broadly from the new foods offered, although, crucially, his preferences changed and cycled over time. It is certain that the proportion of calories derived from sucrose and starch drastically decreased in favor of calories from leaves, lower-sucrose fruits, animal-sourced protein, and oils and fats.

There was also gradual improvement in the condition of the mantled howler monkeys. Two weeks after dietary changes had been implemented, we ensured that they received a wider variety of cultivated greens, appropriate fruits and browse by delivering them personally. Subsequently, they exhibited marked improvement. A few months later,
the old diet gradually replaced the suggested one, and the animals' condition again deteriorated. Once again, dietary changes were implemented, with marked improvement.

Conclusions

Like most in the field, we are aware of numerous instances in which primates are fed diets substantially different from those to which they evolved and require, and on which they exhibit accompanying chronic health problems. Dismaying often, caretakers attempt to resolve the problems with medications, rather than looking to the obvious cause.

The principles we describe for dramatically improving the health of ailing primates are not new; they are described in publications such as the AZA NAG fact sheet on feeding folivorous primates (Edwards, 1997). However, even in such recommendations, which caution against feeding rapidly fermentable carbohydrates, there is great reliance on “high fiber” biscuits, which are very high in just such carbohydrates, and are missing, or low in, a vast array of other phytochemicals (including, despite their names, fiber; see, e.g., the discussion in Lehr et al., 1997), compared to browse. Although we are aware of no reliable data, it would appear that a large fraction of captive primates fed biscuits or biscuits-plus-produce exhibit significant health problems.

Provision of solely authentic foods, in sufficient amounts and variety that toxicities or deficiencies are unlikely, does not seem to be common practice. We are aware of possible problems with relying on local browse or browse fed to other species (e.g., Toddes et al.), but providing a wide array of foliage (cultivated, ornamental, and uncultivated) with established safety should minimize risk. We hope that the observations presented here will promote careful case studies, in which the actual dietary intake of ill primates is analyzed; changes to authentic foods are instituted (either gradually or with the aid of probiotic inoculation), again with actual intake measured and analyzed; and outcomes critically assessed.

Literature Cited


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Appendix: Example of Guideline Chart for Commissary Staff

Captive Siamang Diet

Daily diet by weight (as fed):
50% Plant/green food
30% Fruit
10% Nuts/ seeds
10% Animal-sourced food

Based on 1000 g/day:

<table>
<thead>
<tr>
<th>Item</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 cups loosely packed Plant/green</td>
<td>500 g</td>
</tr>
<tr>
<td>1 ½ cups cut fruit</td>
<td>300 g</td>
</tr>
<tr>
<td>1 cup Nuts/ seeds</td>
<td>100 g</td>
</tr>
<tr>
<td>1 chicken portion + 1 egg w/shell</td>
<td>100 g</td>
</tr>
<tr>
<td>or (1 chicken portion + 2/3 cup kibble)</td>
<td></td>
</tr>
<tr>
<td>or (1 egg w/shell + 2/3 cup kibble)</td>
<td></td>
</tr>
<tr>
<td>or (1 chicken portion + 500 meal worms)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1000 g</td>
</tr>
</tbody>
</table>

General Food Weights

<table>
<thead>
<tr>
<th>Item</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 cups loosely packed fresh greens</td>
<td>50 g</td>
</tr>
<tr>
<td>3 cups tightly packed fresh greens</td>
<td>100 g</td>
</tr>
<tr>
<td>1 cup cooked greens</td>
<td>100 g</td>
</tr>
<tr>
<td>½ cup cut fruit</td>
<td>100 g</td>
</tr>
<tr>
<td>1 small banana</td>
<td>100 g</td>
</tr>
<tr>
<td>1 small apple</td>
<td>100 g</td>
</tr>
<tr>
<td>1 cup nuts</td>
<td>100 g</td>
</tr>
<tr>
<td>1 prepackaged chicken portion</td>
<td>50 g</td>
</tr>
<tr>
<td>1 egg</td>
<td>50 g</td>
</tr>
<tr>
<td>500 mealworms</td>
<td>50 g</td>
</tr>
<tr>
<td>2/3 cup kibble</td>
<td>50 g</td>
</tr>
</tbody>
</table>
AN OVERVIEW OF THE NATIONAL ACADEMIES NEW REPORT:
NUTRIENT REQUIREMENTS OF NONHUMAN PRIMATES

Mark Edwards, Ph.D. and Charlotte Kirk Baer, M.S.

1 Zoological Society of San Diego
2 The National Academies

Introduction

More than 250 species and more than 600 subspecies are recognized in the order Primates. Over 500,000 primates live in biomedical research laboratories and conservation institutions throughout the world. Records of regional primate research centers indicate that about 20,000 nonhuman primates of 28 species were housed in eight U.S. centers in 1999. Records of the International Species Information System indicate that over 9,500 nonhuman primates of 145 species were in U.S. and Canadian zoos at the end of the year 2000. The challenge of describing the nutritional needs of these primates is great because studies of feeding ecology, gastrointestinal anatomy, and nutrient requirements have been completed for only few.

The National Academies new report, Nutrient Requirements of Nonhuman Primates (2003), which is a thorough review of nutrient needs of primates, is one of a series on animal nutrition issued by The National Academies Board on Agriculture and Natural Resources. It was prepared by the Ad Hoc Committee on Nonhuman Primate Nutrition and is a revision of the Academy’s 1978 edition of Nutrient Requirements of Nonhuman Primates.

Preparing the Report

In preparing this new report, the committee of experts appointed by the National Academies was limited in the amount of reliable and specific information available on nutrient requirements, deficiencies, and toxicities in primates. The authors of this publication had as their primary objective the development of guidelines that would ensure that nutrient deficiencies or toxicities and inappropriate dietary husbandry would not limit success in primate research colonies or zoos.

Throughout the 3-year study process, input from others was sought by posing specific questions in widely distributed correspondence, by hosting workshops and information-gathering sessions, and by inviting sponsors of the study and the general public to attend meetings of the committee. Information published before 1978 was reevaluated, that in newer publications was examined, and both have been used along with other input to develop this new report.

Important Features of the Comprehensive Review

This new edition places greater emphasis than before on descriptions of natural dietary habits, gastrointestinal anatomy and physiology, and the special nutrient and dietary husbandry needs of species that traditionally have been difficult to maintain in captivity. Information on nutrient requirements is presented for “model species” in this revision in the hope that such data are representative of the Order. Detailed information on the contents of each chapter, which appears in the report’s Preface, is provided below.
Chapter 1 is a new feature that was not provided in the previous edition. This chapter is intended to give the reader an understanding of variations in feeding ecology and digestive strategies among primates, which is critical knowledge needed to make informed decisions on feeding primates. The discussion is concerned with foraging strategies in natural ecosystems, species differences in gastrointestinal morphology and physiology, and the significance of these factors in development of appropriate systems of dietary husbandry for captive primates. Because the usefulness of data gathered in field studies of feeding ecology varies with the method used, the report discusses the strengths and weaknesses of the methods. Relevant field-study data are tabulated by species, and the various gastrointestinal types found among nonhuman primates is illustrated.

Chapter 2 is a detailed review of energy terms, methods used to determine energy requirements, and energy requirements of nonhuman primates for adult maintenance, growth of young, and pregnancy and lactation. Tables include data on body weight, measured energy expenditures, and estimates of daily metabolizable-energy requirements as multiples of basal metabolic rate. Chapter 3 discusses first the classification of carbohydrates, their characteristics, digestion, metabolism, and analysis and then discusses analytic systems for fiber, the role of dietary fiber in primate gastrointestinal health, and potentially beneficial dietary fiber concentrations. Chapter 4 covers proteins, protein sources, and methods of assessing protein quality and requirements. Information on protein-calorie malnutrition and on protein deficiencies and excesses is included. Although quantitative requirements of nonhuman primates for specific amino acids could not be defined, evidence of the essentiality of methionine, lysine, phenylalanine, tryptophan, and taurine is presented. Protein requirements, based on high-quality reference proteins and various criteria, are given in tabular form. Chapter 5 addresses fats and fatty acids, including classification, nomenclature, digestion, absorption, and metabolism. It describes essential fatty acids and presents estimated requirements for n-3 and n-6 fatty acids. Fatty acid composition of primate milks, potentially harmful fatty acids, cholesterol metabolism, and use of nonhuman primates as models for study of cardiovascular disease are discussed.

Perhaps the most greatly expanded chapters in this revision is Chapter 6, which is a review of mineral nutrition and metabolism, including functions and signs of mineral deficiencies and excesses. In the first edition of this report, which was published in 1978, there was no discussion of sulfur, copper, cobalt, or molybdenum needs of nonhuman primates. This second edition provides the first recommendations on mineral requirements for copper and selenium based on a comprehensive review of the scientific literature. Similarly, Chapter 6 provides the first review and discussion of sulfur and cobalt in primate nutrition by the National Academies. Mineral requirements of several primate species at various ages are given. Chapter 7 is a discussion of fat- and water-soluble vitamins, including form, function, metabolism, and signs of deficiency and toxicity. Estimates of quantitative requirements of nonhuman primates are provided. Chapter 8 deals with water as a component of the primate body and with the influence of activity and various environmental factors on the proportion of body water. Water sources, water quality, water turnover, water requirements, and important considerations in providing water for nonhuman primates are discussed. Chapter 9 presents information on a number of pathophysiologic and life-stage considerations that are relevant to nonhuman-primate nutrition. It includes values of body mass (weight) and body composition, studies of the nutritional needs of neonates, effects of aging on nutritional needs, and relationships of nutrition.
to aging, obesity, and diabetes. Chapter 10 discusses primate-diet formulation, effects of feed processing on nutrient loss, factors that influence food intake, and some general suggestions for dietary husbandry. Plants that have been safely used as browse offerings in captivity are listed.

Providing much more detailed and focused recommendations than the general recommendations provided in the previous edition, Chapter 11 tabulates estimated nutrient requirements of model nonhuman primates in six categories (suborder Strepsirrhini; families Hominidae and Pongidae, Cercopithecidae, Cebidae, and Callitrichidae; and subfamily Colobinae). These requirements were estimated on the basis of a thorough review of the world’s scientific literature, input from numerous scientific sources, and the subcommittee’s best judgment. The requirements apply most satisfactorily to purified diets with high nutrient bioavailability and without substantial adverse interactions among nutrients. The estimates represent minimal requirements without safety allowances. Also provided in this chapter is a table (Table 11-2) of dietary nutrient concentrations proposed as a guide for formulation of diets containing natural ingredients and intended for post-weaning primates. These have been expressed per unit of dietary dry matter, assuming an energy density of 4 kcal ME·DM g⁻¹. It should be noted that these nutrient concentrations are intended only as guides, have not been directly tested as a group with any primate, and may not be appropriate for all species or all post-weaning physiologic stages.

Chapter 12 provides tables of the compositions of feeds commonly used in nonhuman-primate diets. Chapter 13 discusses food as a component of environmental enhancement, an application arising from concern for the psychologic well-being of nonhuman primates in captivity. Various food choices and means of presentation are suggested. The Appendix contains a scheme of taxonomic relationships within the Primate Order, including scientific and common names, plus tables of weight equivalents and weight-unit conversion factors.

Summary

Appropriate dietary husbandry is basic to health, reproductive success, and longevity of nonhuman primates in captivity. This implies provision of both qualitative and quantitative nutrient requirements in dietary forms that are acceptable and that encourage normal eating behavior. In the approximately 25 years since the National Academies first published on nutrient requirements of nonhuman primates, hundreds of relevant studies have been conducted and published. The National Academies, through its independent, objective studies, produces the world’s gold standard for identifying, assessing and providing recommendations based on published research that contributes significantly to animal well-being.

References

In June 2003, Brookfield Zoo’s *Macropus frugilinosus*, western grey kangaroo, mob numbered ten males and six females, all housed in the Australia House exhibit. These animals are fed as a mob and additionally graze on plants in their enclosure. At the time of this study, all but one individual was an adult; the exception was a sub-adult male weighing 6.22 kg. Weights were not available for all individuals, but were estimated based on the weights of those individuals already behaviorally trained to using the scale and known species averages. Adult males can weigh up to 82 kg and females 32 kg (The Kangaroo Center website). This study reviews the diet of these kangaroos as a mob.

The framework for “formulating diets for captive exotic animals” outlined in the 1997 paper of the same name by Dr. Sue Crissey and standard Zoo Nutrition Services procedures at the Brookfield Zoo were followed during this study. Components of the study included foraging ecology of *Macropus frugilinosus* in the wild, digestive anatomy and physiology, nutrient requirements, environment of the captive population, then diet composition established by an intake study with accompanying nutrient analysis, and finally recommendations for diet based in part on the nearest domestic animal model, the horse.

The mob’s daily diet consisted of ZNN herbivore pellets, apples, bananas, carrots, sweet potatoes, Brookfield Zoo leafy mix, and grass hay. They consumed over 95% of the bananas and apples offered, as well as, the corn leaving the cobs. On the other hand, they consumed on average only 52.4% grain, 50.0% grass hay, and 42.7% leafy mix. Overall the diet matter consumed exceeded all probable nutrient requirements. Each nutrient was considered in turn and although none were at toxic levels, the new diet recommended a twenty-five percent decrease overall in quantity and a further decrease in the ZNN herbivore pellets, while still meeting the daily required nutrient and caloric intake for the mob.
FODDER TREES AND BROWSE FOR MEAT GOATS

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Study 1. White mulberry (Morus alba) and three leguminous tree species, Black locust (Robinia pseudoacacia L.), Mimosa (Albizia julibrissin Durazz) and Honey locust (Gleditsia triacanthos), were evaluated for growth, leaf biomass, nutritive value and browsing preference by yearling crossbred Boer goats (Capra hircus hircus). One-year-old seedlings were planted in March 1995. Based on data taken in September 1997 and May 1998, Robinia pseudoacacia (560 and 1,493 kg dry matter [DM]/ha) and Albizia julibrissin (773 and 608 kg DM/ha) produced more leaf biomass than the two other species. Morus alba did not produce as much herbage (243 and 258 kg DM/ha) as the other two species, but was highly preferred by goats. Goats exhibited an initial low preference for Albizia julibrissin but readily consumed that species following defoliation of the other three tree species. Crude protein and neutral detergent fiber concentrations, and in vitro true DM disappearance of leaf samples averaged, respectively: 23, 31 and 96% for Morus alba; 23, 44 and 60% for Robinia pseudoacacia; 24, 33 and 84% for Albizia julibrissin; and 18, 43 and 71% for Gleditsia triacanthos. Although of good quality and readily consumed by goats, Gleditsia triacanthos was judged to be a low value browse species due to its low biomass production (98 and 172 kg DM/ha). Subsequent leaf production data of Robinia pseudoacacia by hand defoliation of the same trees twice during the growing season indicated that that species produced large amounts of herbage (June 2001: 1,682 kg DM/ha; August 2001: 4,060 kg DM/ha; June 2002: 5,580 kg DM/ha; September 2001: 2,043 kg DM/ha). These results indicate that Robinia pseudoacacia, Albizia julibrissin and Morus alba have high potential as silvopastoral species and could potentially play an important role in meat goat production systems. Nevertheless, the importance of anti-quality factors such as tannins, that decreased in vitro true DM disappearance of Robinia pseudoacacia, will have to be evaluated in in vivo experiments. Conversely, tannins present in Robinia pseudoacacia may represent a useful alternative to traditional anthelmintics to control gastrointestinal parasitic worm loads in goats.

Study 2. A 4-year field study was initiated to evaluate the effectiveness of rotationally grazing crossbred Boer goats in combination with cattle (GC; 0.3 ha/goat and 0.6 ha/steer) or cattle (Bos taurus) alone (C: 0.6 ha/steer) to manage vegetation in an abandoned orchard (8.4 ha) that had not been grazed for two years. Robinia pseudoacacia trees were practically eliminated over the 4-year period in both C and GC pastures ($P < .01$) but grew to a height of 5.3 m in the control (CTL). Height of Multiflora rose (Rosa multiflora Thunb.) bushes were controlled in GC (0.5 m) but increased to 1.8 m in C and 2.5 m in CTL (C vs GC: $P < .02$; CTL vs C + GC: $P < .01$). Similarly, Rosa multiflora canopy area was controlled in GC (0.4 m²), but increased in C (from 0.6 to 7 m²; $P < .01$) and greatly increased in CTL (from 0.5 to 11 m²; $P < .01$). The cattle provided only modest control of Rosa multiflora. Inclusion of goats resulted in a reduction of live canes of Rosa multiflora at the conclusion of the study relative to other treatments (CTL: 95%; C: 96% GC: 41%). Honeysuckle (Lonicera japonica T.) was practically eliminated in
grazed pastures (avg frequency: 5.7%) but increased to 52% in CTL at the conclusion of the study (P < 0.01). Vegetative ground cover decreased in CTL (from 80 to 66%; P < .01) but was similar (avg 90%) in the C and GC pastures. Similarly, cover from herbaceous grass species decreased in CTL (78 to 40%; P < .01) but remained similar in C and GC (avg 81%). The chemical composition of the plant browsed by the goats (Table 1) indicated that their quality was sufficient to meet the nutritional requirements of young growing goats. Results indicated that controlled grazing improved mountain pastures, and that grazing goats with cattle was especially beneficial for the control of Rosa multiflora bushes and other browse species. In addition to providing needed nutrients, fodder trees and other browse plants also provide natural shade that helps young, actively growing meat goats mitigate environmental stresses.

Table 1. Chemical composition (%) of various plants browsed by goats

<table>
<thead>
<tr>
<th>Browse type, including leaves and petioles</th>
<th>Crude protein</th>
<th>Neutral detergent fiber</th>
<th>Calcium</th>
<th>Phosphorous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosa multiflora</td>
<td>18.2</td>
<td>34.5</td>
<td>0.99</td>
<td>0.32</td>
</tr>
<tr>
<td>Robinia pseudoacacia</td>
<td>23.0</td>
<td>44.0</td>
<td>1.26</td>
<td>0.21</td>
</tr>
<tr>
<td>Lonicera japonica</td>
<td>16.0</td>
<td>34.5</td>
<td>1.21</td>
<td>0.30</td>
</tr>
<tr>
<td>Rubus spp</td>
<td>17.1</td>
<td>24.5</td>
<td>0.23</td>
<td>0.84</td>
</tr>
<tr>
<td>Ligustrum vilgare</td>
<td>20.0</td>
<td>26.8</td>
<td>0.89</td>
<td>0.34</td>
</tr>
<tr>
<td>Smilax rotundifolia</td>
<td>16.1</td>
<td>39.5</td>
<td>0.60</td>
<td>0.18</td>
</tr>
<tr>
<td>Campsis radicans</td>
<td>16.7</td>
<td>43.1</td>
<td>0.42</td>
<td>0.22</td>
</tr>
</tbody>
</table>

**Study 3.** A multi-year study was initiated in summer 2003 to evaluate the effectiveness of using goats to control kudzu (*Pueraria lobata*). Following weaning, twenty mature crossbred Boer does that had kidded in late February – early March were browsed on three 0.5 ha plots. Plots were browsed 5 times between 4 June and 8 October. When not browsed on kudzu plots, goats grazed a tall fescue (*Festuca arundinacea*) and bermudagrass (*Cynodon dactylon*) pasture. Goats readily consumed all kudzu leaves and terminal stems. Crude protein (CP) concentrations of kudzu leaves prior to the first and subsequent grazing cycles were 24% (June), 22% (July), 23% (August), 24% (September), and 24% (October). The CP concentrations of kudzu leaves that had not been grazed throughout the grazing season were still 24% in early October. Edible stems sampled in June had CP concentrations of 12%. Body condition of the does and herbage chemical composition indicated that kudzu is a high quality feed source for browsing ruminants from late spring until early fall.
INTAKE, DIGESTIBILITY, AND NITROGEN UTILIZATION OF BLACK LOCUST FOLIAGE FED TO GROWING GOAT WETHERS

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In southeastern USA, goats (Capra hircus hircus) are becoming an important livestock species due to increased demand for goat meat by various ethnic groups. Goat meat also fills a gap in some high-value niche markets for people who prefer low levels of total and saturated fat in their diet. For the past decade, the domestic market has not been able to supply these increasing demands. An important consideration in increasing meat goat production is the availability of sustainable production systems. Black locust (BL; Robinia pseudoacacia L.) is indigenous to Southern Appalachian deciduous forests. It is an appropriate source of biological nitrogen (N) that can be used to enhance N-poor ecosystems, control erosion, and provide potential browse for livestock.

Black locust leaves have been used for livestock feed around the world, including Korea and Bulgaria. In the highlands of Nepal and northern India, where BL is naturalized, it has been documented that branches above the reach of livestock are cut and fed to livestock when other green forages are scarce, and the wood is used for fuel. Black locust has been grown successfully in dry Mediterranean climates to effectively satisfy feed demand when grasses and legumes become dormant in the summer. Furthermore, researchers have reported that goats have a high preference for BL as browse.

Black locust leaf N levels have been reported to be similar to those of alfalfa, ranging from 3.2 to 4.0%. Research also showed that BL accumulates moderately high macronutrient concentrations (1.27% Ca, 1.72% K, and 0.18% P) and produces ample foliage with high leaf surface area, rendering this species an excellent browse source for meat goat production.

Phenolic compounds (e.g. tannins) occur in BL, acting as chemical defenses against herbivory. The presence of tannins is hypothesized to negatively affect intake and digestibility of foliage. The high tannin concentrations contained in some forages and browse can have adverse effects on the nutrition of grazing animals. Tannins bind to proteins and form an insoluble protein-tannin precipitate that is poorly digestible in the rumen and the lower digestive tract and is excreted in the feces. Tannins also can inhibit microbial enzymes used in fiber breakdown. Nonetheless, there is evidence that dietary tannins have some potential benefits such as acting directly as natural anthelmintics against parasitic nematodes, indirectly improving N supply, and preventing bloat.
In 1999 and 2000, BL was fed to sixteen, four month old crossbred Boer wether goats (20.4 kg initial body weight). Four diets were stall-fed in a randomized complete block design with four replications in each of two years. The objective of this research was to study the effect of feeding BL foliage on intake, digestibility, and N metabolism. In study 1, diets were eastern gamagrass hay [EGH; Tripsacum dactyloides], 70% EGH and 30% mixture of 59% ground corn [GC; Zea mays], 36% soybean meal [SBM; Glycine max], and 5% minerals), 75% EGH and 25% BL leaves), and 50% EGH and 50% BL leaves). These diets respectively contained 2.18, 2.83, 2.91 and 3.25% N. Diets fed in study 2 were orchardgrass hay [OGH; Dactylis glomerata L.], 70% OGH and 30% mixture of 63% GC and 37% SBM), 50% OGH and 50 % BL leaves), and 25% OGH and 75% BL leaves). The N concentrations of these diets were, respectively, 2.48, 2.8, 3.62, and 3.95%. Black locust foliage concentrations of condensed and hydrolysable tannins were, respectively, 10.4 and 34.2% in study 1, and 10.3 and 18.7% in study 2.

In study 1, goats consumed similar amounts of DM across all four diets whereas in 2000 DM intake was lower for the diet containing 75% BL leaves compared to the 50% BL leaf diet (P = 0.04). Inclusion of BL foliage decreased cell-wall digestibility in both trials (P < 0.01). Nitrogen retention as a percentage of N intake decreased in the BL-containing diets compared to the EGH + grain diet (P < 0.08), whereas fecal N excretion as a percentage of N intake increased (P <0.01). Conversely, urinary N excretion as a percentage of N intake remained constant. Total volatile fatty acids (VFA) were not affected whereas ruminal NH$_3$-N concentrations were lower in the BL-containing diets compared to the EGH + grain diet (P < 0.01).

In study 2, N retention as a percentage of N intake was similar across diets whereas fecal N excretion as a percentage of N intake was higher in the BL-containing diets compared to the OGH + grain diet (P < 0.01). Urinary N excretion as a percentage of N intake was lower in the BL-containing diets compared to the OGH only and the OGH + grain diets (P < 0.02). Total VFA and ruminal NH$_3$-N concentrations were reduced (P < 0.01) in goats fed the BL diets.

Increased levels of BL in the diets increased fecal N, which suggested that tannins formed dietary protein complexes, and hindered digestibility of cell wall constituents. Nevertheless, nitrogen balance data in study 2 seem to indicate that the dietary N levels in the BL-containing diets were high enough to counterbalance the negative effects of tannins. If intake of this tannin rich browse species can be controlled to keep tannin intake within limits that imply beneficial effects, use of BL could be a promising alternative forage in the Southeastern USA.

Additional research is warranted to examine meat goat performance under grazing situations where animals would have free access to a greater variety of herbaceous plants to dilute and possibly counter-balance the negative effects of tannin compounds found in BL, while taking advantage of the low fiber and high CP concentration found in this browse species.
A DIET SURVEY SUMMARY OF CAPTIVE DIETS CONSUMED AND STOOL QUALITY OF BEARS

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In order to determine the nutrient requirements of an animal, it is necessary to conduct intensive nutrient balance studies. It is nearly impossible to conduct these types of studies with captive exotic animals. Quantification of nutrient levels consumed by healthy animals can be used to help formulate recommendations. Through multi-institutional surveys, it is possible to collect a broad range of background information. Because the nutrient content of the diet consumed is not always the same as the nutrient content of the diet offered, a survey should consist of an intake study and a questionnaire. It is also advantageous to have several institutions participate in order to increase sample size.

The Fort Worth Zoo conducted a survey, as an AZA Bear TAG project, that included 40 zoos, 7 bear species and over 100 bears. Data were compiled about the diets offered to various species of bears and the actual intake of these diets. The nutrient composition of the diet was calculated and used to compare dietary intake to conditions such as stool color and consistency, body and skin condition, energy levels and particular behaviors. There was a large amount of variation in the data and statistical analysis was not possible, however, some trends were seen. All bears appeared to be healthy. Polar bears had the best stool quality and black bears had the poorest stool quality (Table 1). Higher fat appeared to be correlated with better stool quality in most of the bear species (Table 1). In general, canine complete feeds resulted in better stool quality when compared to omnivore, polar, and primate complete feeds. Some consumed protein levels were low compared to known requirements for dogs and cats (NRC, 1986; AAFCO, 1991), but were similar to the minimal dietary requirements for wild black and grizzly bears (Rode and Robbins, 2000) (Table 1).

Literature Cited:


Table 1: Bear TAG Consumption Study: Nutrient Intake and Stool Condition Summary

<table>
<thead>
<tr>
<th>Bear Species</th>
<th>Stool Grade</th>
<th>N</th>
<th>Protein</th>
<th>Fat</th>
<th>Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polar</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>16±1.5</td>
<td>6.9±1.6</td>
<td>0.5±0.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>20±2.3</td>
<td>11±3.9</td>
<td>1.7±0.5</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>1</td>
<td>21</td>
<td>10</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>20</td>
<td>8.6</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td><strong>Asiatic Black</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>9±2.4</td>
<td>2.8±0.9</td>
<td>1.6±0.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>19±0.4</td>
<td>8.9±0.1</td>
<td>2.2±0.2</td>
<td></td>
</tr>
<tr>
<td><strong>American Black</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>24±1.0</td>
<td>13±0.6</td>
<td>1.8±0.1</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>6</td>
<td>18±3.3</td>
<td>6.3±1.4</td>
<td>4.6±0.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>16±2.4</td>
<td>6.0±1.6</td>
<td>2.0±0.5</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>2</td>
<td>22</td>
<td>7.1</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td><strong>Kodiak</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>18±1.7</td>
<td>8.8±0.5</td>
<td>1.4±0.3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>14±3.5</td>
<td>5.7±1.3</td>
<td>2.2±0.6</td>
<td></td>
</tr>
<tr>
<td><strong>Grizzly</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>14</td>
<td>6.5</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>4</td>
<td>12±4.0</td>
<td>6.0±0.1</td>
<td>1.8±0.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>10±2.9</td>
<td>4.4±2.1</td>
<td>1.6±0.3</td>
<td></td>
</tr>
<tr>
<td><strong>Spectacled</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>8.4±2.6</td>
<td>5.3±3.8</td>
<td>2.9±1.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>15±5.4</td>
<td>5.4±0.9</td>
<td>2.6±0.8</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>7</td>
<td>17±4.9</td>
<td>5.9±0.6</td>
<td>2.5±0.7</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>15±4.0</td>
<td>4.2±0.8</td>
<td>2.0±0.4</td>
<td></td>
</tr>
<tr>
<td><strong>Sun</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>12±0.3</td>
<td>4.9±0.6</td>
<td>2.0±0.2</td>
<td></td>
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<tr>
<td>3</td>
<td>4</td>
<td>16±1.7</td>
<td>4.9±1.2</td>
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<td></td>
</tr>
<tr>
<td>3.5</td>
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<td>2.3±0.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>9.7±5.5</td>
<td>2.9±1.5</td>
<td>1.4±0.5</td>
<td></td>
</tr>
<tr>
<td><strong>Sloth</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>2</td>
<td>23</td>
<td>7.5±0.2</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>25</td>
<td>6.7</td>
<td>2.8</td>
<td></td>
</tr>
</tbody>
</table>

1 Food offered and remaining collected over 4 days to calculate nutrient levels consumed by bears.

2 The above values are NOT suggested guidelines. In general, guidelines are generated considering a critical evaluation of these data as well as a review of known nutrient requirements for species with similar feeding habits.

3 Stool grading system: 1 – dry, crumbly; 2 – well formed, does not leave a mark, easy to pick up (even from grass); 3 – slightly moist, less well-formed, leaves a mark when removed, tacky to the touch, soft centered; 4 – moist, badly formed, consistency of porridge/putty; 5 – diarrhea.
EVALUATION OF NUTRITIONAL CONDITION IN THE FRESHWATER MUSSEL, ELLIPTIO COMPLANATA

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4North Carolina State University, Department of Environmental and Molecular Toxicology
5North Carolina State University, Department of Zoology

Abstract

We tested hemolymph and physical parameters of Elliptio complanata subjected to three different algae feeding levels in a laboratory experiment designed to evaluate potential indicators of nutritional imbalance. After three months at markedly different algae feeding rates, only small differences in standard physiologic parameters developed between the treatment groups. Foot tissue glycogen values were not significantly different between treatment groups upon completion of the experiment. Contrary to expectations, the highest foot glycogen values were actually recorded in the low-feed treatment group. Hemolymph calcium, magnesium and phosphorus did show statistically significant, though small, differences by feeding level. Hemolymph glycogen also registered small statistically significant gradations in concentration relative to feeding rates. Stable nitrogen isotope analysis, showed enrichment in foot tissue $\delta^{15}N$ with reducing feed levels. Stable carbon isotope analysis found the reverse trend, with the most enriched $\delta^{13}C$ values in the high feed groups suggesting that the algal food supplied was not the main source of nutrition during the studies.
Table 1: Treatment medians and significance tests for parameters measured at the end of the experiment. Sample sizes are n=10 for each treatment group unless otherwise specified.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low-feed Median</th>
<th>Moderate-feed Median</th>
<th>High-feed Median</th>
<th>Jonckheere Test P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot δ¹³C, per mil</td>
<td>-31.36 (n=9)</td>
<td>-29.729</td>
<td>-28.32 (n=9)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Magnesium</td>
<td>1.4 mg/dL</td>
<td>1.65 mg/dL</td>
<td>1.85 mg/dL</td>
<td>0.0029</td>
</tr>
<tr>
<td>Cell Glycogen (mg per dL hemolymph)</td>
<td>1.821 mg/dL</td>
<td>5.503 mg/dL</td>
<td>5.513 mg/dL</td>
<td>0.0113</td>
</tr>
<tr>
<td>Foot δ¹⁵N, per mil</td>
<td>7.962 (n=9)</td>
<td>7.347</td>
<td>7.062 (n=9)</td>
<td>0.0166</td>
</tr>
<tr>
<td>Calcium</td>
<td>16.45 mg/dL</td>
<td>17.9 mg/dL</td>
<td>18.4 mg/dL</td>
<td>0.0287</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.5 mg/dL</td>
<td>0.5 mg/dL</td>
<td>0.6 mg/dL</td>
<td>0.0495</td>
</tr>
<tr>
<td>Ammonia</td>
<td>&lt;10 umol/L</td>
<td>&lt;10 umol/L</td>
<td>14 umol/L</td>
<td>0.0516</td>
</tr>
<tr>
<td>Weight-to-Volume Ratio, mg/mm³</td>
<td>0.633</td>
<td>0.666</td>
<td>0.651</td>
<td>0.0594</td>
</tr>
<tr>
<td>Hemolymph δ¹⁵N, per mil</td>
<td>8.227</td>
<td>8.045 (n=7)</td>
<td>7.551 (n=8)</td>
<td>0.0808</td>
</tr>
<tr>
<td>Change in Weight (gain)</td>
<td>0.58 g</td>
<td>1.33 g</td>
<td>1.34 g</td>
<td>0.1521</td>
</tr>
<tr>
<td>Glucose</td>
<td>1 mg/dL</td>
<td>1 mg/dL</td>
<td>2 mg/dL</td>
<td>0.1660</td>
</tr>
<tr>
<td>Foot Glycogen</td>
<td>72.98 mg/g</td>
<td>60.85 mg/g</td>
<td>67.89 mg/g</td>
<td>&gt; 0.500</td>
</tr>
</tbody>
</table>
METABOLIC BONE DISEASE IN CAPTIVE-REARED LOGGERHEAD SEA TURTLE HATCHLINGS

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2Environmental Medicine Consortium and Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606; pamgovett@hotmail.com

In 2002, over 400 loggerhead sea turtle (Caretta caretta) hatchlings were reared in North Carolina, and over 700 in Florida for an empirical study of sex ratios (Wyneken et al. 2003). Unlike most previous studies of sea turtle hatchling sex ratios, which utilize post-mortem histology of the gonad, this study was designed to determine sex nonlethally. By rearing hatchlings from their emergent size of approximately 20 g until they reach 120 g and up, sex can be determined by laparoscopic examination and biopsy, after which turtles can be released. Based on a desire for rapid short-term growth and prior experience, investigators elected to feed a diet of chopped shrimp tail meat (Alexander 2000). Turtles were raised indoors, in individual containers held within larger tanks of seawater at 24 - 28 °C, under 12D:12L broad spectrum lighting, though the UV irradiance at water surface was later measured at the North Carolina site and determined to be minimal. While the turtles initially met expectations for rapid growth, as they exceeded 60 g, a proportion of each colony began exhibiting clinical signs consistent with metabolic bone disease. These included a soft, pliable mandible and skull, as well as an excessively flexible shell (recognizing that sea turtle hatchling shells naturally are less rigid than many of their freshwater and terrestrial counterparts), wide seams between plastron scutes, reduced feeding, and weight loss. In severe cases, the plastron became concave and folded, the skull was easily deformed by gentle pressure exerted with thumb and fore-finger. In one terminal case generalized tremors were observed.

Plasma calcium and phosphorus measurements on two affected turtles were as follows: Ca 2.89 and 3.24 mg/dl, and P 13.99 and 14.08 mg/dl. Follow-up Ca and P measurements on seven additional grossly affected and unaffected turtles were Ca 4.8, P 8.3; Ca 4.2, P 9.1; Ca 3.4, P 12.7; Ca 5.0, P 10.0; Ca 4.3, P 10.9, Ca 3.6, P 10.7; and Ca 3.8, P 8.7. While mildly inverted plasma Ca/P ratios are commonly encountered in apparently healthy wild loggerhead sea turtles, the ratios of the captive hatchlings ranged from 0.21 - 0.56, or approximately 1:5 to 1:2. Interestingly, some turtles fed the shrimp tail meat diet in Florida developed similar shell deformities, but did not have the markedly inverted plasma Ca: P ratios. Histologic findings on one mortality included retained cartilaginous cores, osteopenia and fibrous osteodystrophy of the humerus, fibrous osteodystrophy of the skull, and hyperplasia of the parathyroid gland.
Some seriously-affected turtles were treated individually with variable success by tube feeding slurries of a gel diet or Hills A/D, administering parenteral calcium chloride and Vitamins A/D, and systemic antibiotics. Recognizing that the problem had to be addressed on a herd basis to succeed, however, emphasis was placed on modifying the diet for the entire collection. Because many turtles had formed dietary preferences, multiple approaches were incorporated, including a commercial gel food (Mazuri®), an in-house gel food modified from Stamper & Whitaker (1994), whole mysid shrimp, small live crabs captured locally, and outdoor sunlight swims as weather permitted. As time progressed, most turtles were converted to the in-house gel diet. Behavioral responses to live crab feeding were strongly positive by both the turtles and their caretakers. Previously-affected turtles that transitioned to more complete diets grew well and their skeletal structures became more robust, while fewer new cases emerged. The roles of calcium and phosphorus absorption from the water and fluctuating salinity in the estuarine water supply in affecting the captive sea turtle calcium and phosphorus balance are undetermined. Mineral content data on whole fish and marine invertebrates published by the AZA Nutrition Advisory Group (Bernard & Allen 1997) were critical to making recommendations for dietary modifications.

This year over 200 turtles are being reared in North Carolina and 290 in Florida. Turtles are fed initially on mysid shrimp, and transitioned to the in-house gel diet. Growth has been slower this year at the northern site, requiring a one month delay in the first laparoscopy compared with last year, but only one turtle has exhibited signs of metabolic bone disease in North Carolina and none in Florida. This turtle refused the gel diet, and readily consumed only mysid shrimp for an extended period.

Literature Cited


UROLITHIASIS IN CAPTIVE GIRAFFE (GIRAFFA CAMELopardalis)

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Since 1998, at least 6 captive giraffe in United States collections have died or have had to be euthanized as a result of the development of obstructive urolithiasis. Review of historical records indicates that perhaps as many as 22 giraffe mortalities since 1966 have been associated with urolithiasis. This would place this condition among the most significant single health risks in adult giraffe in captive collections to date.

Urolithiasis, or urinary calculosis, is the development of concretions within the urinary tract. Obstructive urolithiasis is the accumulation of these calculi, usually in the urethra, restricting or eliminating urine flow. The outcome in most cases is rupture of the bladder, leading rapidly to uremic crisis and death. Urolithiasis is a relatively common condition in domestic ruminants, such as cattle, sheep and goats. In some studies, incidence has been reported to be as high as 50% in feedlot cattle. The condition is most prevalent in adult males, particularly those castrated early in life, due to the restricted size of the urethra as it passes through the fibroelastic penis.

Insight into the causes of urolithiasis begins with the analysis of the calculi: in giraffe cases in the past 4 years, the calculi have been composed primarily of calcium apatite (calcium phosphate) and struvite (magnesium ammonium phosphate). Development of these 'phosphatic' calculi in domestic ruminants has been attributed to dietary and metabolic imbalances in calcium, phosphorus, magnesium, ammonium and protein. According to ISIS, captive giraffe serum phosphorus levels average 10.6 mg/dl (n = 115; normal range for cattle, 5.6-6.5 mg/dl); mean serum calcium levels are 8.1 mg/dl (n=121; normal range for cattle, 9.7-12.4 mg/dl). This may indicate abnormal phosphorus levels and calcium:phosphorus ratios in these animals.

Most giraffe in U.S. collections are fed commercial pelleted feeds as a significant proportion of their diet; mortalities due to urolithiasis have been reported in giraffe fed at least three different pelleted formulations. Concentrated feeds have been implicated as calculogenic for domestic ruminants due to the high phosphorus content and tendency to cause production of urinary mucoproteins, which act as the 'cement' in forming urinary calculi. Furthermore, rapid intake of concentrated feeds and the resulting decrease in the production of buffering saliva can lead to altered rumen and urine pH and excretion of urinary phosphorus, encouraging urolith formation. Rumen pH alterations can also be caused by improper effective fiber content in the feed, as is the case with animals fed high proportions of pelleted diet.

Another contributing cause of urolithiasis in giraffe may be dehydration and urine concentration. Maluf points out that the kidneys of giraffe, natural inhabitants of a very dry climate, have anatomical similarities to those of desert rodents, and that giraffe in the wild can derive the majority of their dietary water from eating succulent acacia leaves (approximately 60% water) in the absence of surface water. This author comments, however, that in drought
conditions, the giraffe kidney may be predisposed to the development of urolithiasis by the concentration of urine. Giraffe also undergo a wider diurnal fluctuation in body temperature than other animals without necessitating evaporative heat loss, thereby further conserving water. Therefore, giraffe in captivity (fed hay and pellets with very low water content) may take in inadequate quantities of water, promoting urine concentration and encouraging the development of calculi.

Based on this information, key contributing factors to urolithiasis in giraffe may be:

- Improper dietary mineral balance
- Increased urine pH, phosphorus and mucoproteins due to pelleted rations or improper fiber intake
- Low water intake and concentrated urine

Recently, a survey of 45 U.S. institutions holding giraffe was conducted by the American Zoo and Aquarium Association (AZA) for its Giraffe Husbandry Manual. This survey provided useful information on the common feeding practices and husbandry methods of many zoological institutions, and is useful in establishing contacts for further study. For instance, while many institutions feed giraffe alfalfa hay and pelleted rations as described, some provide daily access to acacia trees, more closely simulating the animals' natural diet. By following this with a more in-depth survey as well as sampling the feed content of a broad subset of these institutions, including those with histories or clinical evidence of urolithiasis and those without, we hope to gain insight into the dietary factors causing urinary calculosis in giraffe.

A study is under way to investigate the specific causes of urinary calculosis in captive giraffe, first by initiating a comprehensive survey of U.S. collections aimed at giraffe feeding practices and health histories, and supported by specific analysis of feeds, water, serum, urine and feces. By combining the information gained through our survey with results from an experimental phase of the project, we expect to formulate plans for further study aimed at eliminating the inciting factors in giraffe urolithiasis.

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HUSBANDRY AND FEEDING OF THE EARTHWORM, *LUMBRICUS TERRESTRIS*, FOR TOXICOLOGY STUDIES

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²Ecologist NCEA-Washington, Research Triangle Park, NC

Earthworm species are often used in toxicology studies because they are readily available from commercial sources and as representative organisms living in soil. Earthworms are also used as a food source for other classes of animals, primarily amphibians, and can be used in models of bioaccumulation of environmentally important chemicals. Two main classifications of earthworms are used for toxicology studies: anecic and epigeic. Anecic species, such as *L. terrestris*, live in deep permanent burrows and feed from the soil surface. Epigeic species, *Eisenia sp.*, are found in areas with rich organic matter and live in the upper topsoil. Because of the requirement for permanent burrows in order for anecic species to reproduce, they are more difficult to cultivate and there is less available information on their husbandry in the scientific literature. Nevertheless, *L. terrestris*, the common night crawler, is often used as a test species for toxicology studies primarily because they are large, and relatively hardy earthworms. To facilitate use of *L. terrestris* in studies in our laboratory, we found it necessary to develop improved methods for managing and feeding them in a laboratory setting.

Earthworms have been kept successfully for laboratory studies in small polyethylene containers. Several environmental factors need to be considered including temperature, light, moisture and pH of the substrate. Ideally, earthworms are kept at temperatures of approximately 50°C and in the dark to reduce "escapes" from the container. The choice of substrate, or soil, needs to be chosen to allow for the appropriate moisture retention, pH, and to prevent unsuspected chemical contamination. For the purposes of our study, *L. terrestris* have been housed in a commercially available soil (Scott's brand garden soil). This soil retains water well without leading to pooling of water on the bottom of the containment surface. It is important to avoid commercial soils which are mixed with peat moss because hydrophobic nature and low acidity of the peat. A pH of 6.5-7.5 is preferred for raising most earthworm species. [1]

*L. terrestris* is frequently used for acute soil toxicity studies so many investigators have formalized soil: water: feed ratios. Karnak and Hamelink standardized a test medium with 900 grams of soil to 100 milliliters of distilled water. They also studied the effects of adding various proportions of feed per one kilogram of soil. Results showed that the most effective growth in body weight of *L. terrestris* between 25-75g of feed/kg soil. The authors also tested the effectiveness of using different animal manures as feed sources. Among chicken manure, cow manure, rabbit manure, and corn meal, rabbit manure resulted in the greatest growth and survival of the worms. [2] A challenge when considering manure as a food source for earthworms that are to be used in toxicology studies is to find manure from animals that have not been exposed to interfering drugs. Ivermectin, a commonly used
parasiticide used to treat a variety of species, is of particular concern. Studies have shown that concentrations of ivermectin found in the feces of treated animals can cause mortality in earthworms [3] besides having the potential to confound analysis of target chemicals under investigation.

The most successful care and feeding protocol we have used in our laboratory for *L. terrestris* consists of housing the worms in polyethylene containers approximately three-quarters filled with Scott's garden soil and moistened with distilled water according to the ratio from Karnak and Hamelink. [2] These containers are maintained in a refrigerator operating at 50 degrees celcius. Covering the containers with a layer of moistened cheesecloth and aluminum foil perforated with an 18-gauge needle to provide air holes (approximately 4 per square inch) has helped to maintain the appropriate moisture level in the soil. The container can support 70 worms, which are fed 30 grams of rabbit feces every other week by sprinkling the pellets on top of the soil. The rabbit feces are from a source that has not been treated with ivermectin for a minimum of 60 days prior to collection. The average holding times for *L. terrestris* in our study have been 60 days or less so we do not have experience with this method as a long-term maintenance protocol.

**Literature cited:**


NUTRIENT CONSUMPTION AND IRON STATUS IN SEVEN BLACK RHINOCEROS
(DICEROS BICORNIS) IN THREE INSTITUTIONS

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Hemosiderosis occurs in captive black rhinoceros but not in free ranging animals.1,3 Though plants consumed by free-ranging black rhinoceros have been identified, their contribution to the overall diet has not been quantified. Consequently the level of iron consumed by free-ranging animals, as well as other minerals and iron binding polyphenolics, is not known. Diets offered captive black rhinoceros have been quantified.4 However, nutrient levels consumed often differ from levels offered. Nutrient consumption data were collected for a 6 month period for black rhinoceros at the Brookfield Zoo, Fort Worth Zoo, and Fossil Rim Wildlife Center. Serum iron parameters were measured prior to the 6 month period and following.

The ratio of diet ingredients set at each institution was reflective of the diet normally consumed by those animals (Table 1). For the 6 month period diets were manipulated to ensure the set ratios were consumed. Diets were analyzed for crude protein, neutral detergent fiber, acid detergent fiber, fat, calcium, phosphorus, magnesium, potassium, sodium, iron, zinc, copper, manganese, total iron binding polyphenolics (TIBP), and iron binding tannins (IBT) by standard procedures. Blood was collected prior to and post the 6 month period. Ferritin, transferrin saturation, total iron binding capacity (TIBC), and iron were among parameters determined.

All nutrient levels met or exceeded known nutrient requirements for horses,2 suggested as a guide for black rhinoceros. Crude protein, fiber fractions, calcium, magnesium, potassium, and sodium levels appeared similar while concentrations of fat, iron, zinc, copper and manganese varied among institutions (Table 2). No TIBP or IBT were detected in any diet. Comparing iron status parameters pre and post the 6 month period, serum ferritin values increased in all animals except one. It is possible that lack of consistency in the time of day samples were taken pre and post could have been a factor. Transferrin saturation and serum iron decreased or changed little for all animals while TIBC varied (Table 3). Because iron parameters vary based on sex and age, it was not possible to combine all animals at each institution for comparison. In general, the institution with the most animals with low serum ferritin values was also the institution with the lowest iron diet (Fossil Rim). Serum ferritin values for Brookfield animals ranged from the second lowest to the highest across institutions. Across institutions serum ferritin values were within the range reported for long term captive black rhinoceros, 2,200±2,240 ng/ml, but well above the 133±62 ng/ml reported for free-ranging animals.3 Transferrin saturations were also within the range reported for captive animals (65±22 %), with two animals from two different institutions similar to free-ranging values, 28±6 %.3 Serum iron values for most animals exceeded those for free-ranging animals, 101±19 ug/dl.3
This work could not have been completed without the significant contribution of Sue Crissey, Ph.D. Sue was a co-investigator on this project, which is part of a grant, "Dietary iron absorption and the role of tannins in Eastern (Diceros bicornis michaeli) and Southern black rhinoceros (Diceros bicornis minor), a comparison, funded by IRF and SOSRhino.

Literature Cited

Table 1: Ingredient contribution of diets consumed by black rhinoceros at 3 institutions on a dry matter basis.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Brookfield</th>
<th>Fort Worth</th>
<th>Fossil Rim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pellets</td>
<td>45.8 %</td>
<td>39.0 %</td>
<td>48.8 %</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>39.7 %</td>
<td>50.3 %</td>
<td>31.9 %</td>
</tr>
<tr>
<td>Grass Hay</td>
<td>7.8 %</td>
<td>8.3 %</td>
<td>18.3 %</td>
</tr>
<tr>
<td>Produce</td>
<td>6.5 %</td>
<td>2.4 %</td>
<td>0.8 %</td>
</tr>
<tr>
<td>Supplements</td>
<td>0.2 %a</td>
<td>0 %</td>
<td>0.2 %b</td>
</tr>
</tbody>
</table>

*aTrace mineral salt.

*bBonemeal and Missing Link.

Table 2: Nutrient content of diets consumed by black rhinoceros at 3 institutions on a dry matter basis.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Horse Rec.</th>
<th>Brookfield</th>
<th>Fort Worth</th>
<th>Fossil Rim</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein, %</td>
<td>8-15</td>
<td>16.9</td>
<td>15.7</td>
<td>16.5</td>
<td>16.4±0.6</td>
</tr>
<tr>
<td>Neutral Detergent Fiber, %</td>
<td>-</td>
<td>37.7</td>
<td>36.3</td>
<td>36.9</td>
<td>37.0±0.7</td>
</tr>
<tr>
<td>Acid Detergent Fiber, %</td>
<td>-</td>
<td>23.0</td>
<td>22.3</td>
<td>20.7</td>
<td>22.0±1.2</td>
</tr>
<tr>
<td>Crude Fat, %</td>
<td>-</td>
<td>1.6</td>
<td>1.6</td>
<td>2.2</td>
<td>1.8±0.4</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>0.3-0.6</td>
<td>0.8</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.2-0.3</td>
<td>0.6</td>
<td>0.4</td>
<td>0.4</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>Magnesium, %</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3±0.03</td>
</tr>
<tr>
<td>Potassium, %</td>
<td>0.3-0.4</td>
<td>2.1</td>
<td>2.1</td>
<td>1.9</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>Sodium, %</td>
<td>0.1</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3±0.01</td>
</tr>
<tr>
<td>Iron, ppm</td>
<td>50</td>
<td>227.4</td>
<td>414.0</td>
<td>200.0</td>
<td>280.5±116.4</td>
</tr>
<tr>
<td>Zinc, ppm</td>
<td>40</td>
<td>207.7</td>
<td>107.4</td>
<td>73.9</td>
<td>129.5±69.7</td>
</tr>
<tr>
<td>Copper, ppm</td>
<td>10</td>
<td>38.3</td>
<td>44.3</td>
<td>12.7</td>
<td>31.7±16.8</td>
</tr>
<tr>
<td>Manganese, ppm</td>
<td>40</td>
<td>171.0</td>
<td>88.7</td>
<td>88.2</td>
<td>116.0±47.7</td>
</tr>
</tbody>
</table>
Table 3: Iron status parameters in black rhinoceros at 3 institutions.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Ferritin a</td>
<td>1761</td>
<td>2625</td>
<td>2283</td>
<td>2394</td>
<td>550</td>
<td>772</td>
<td>2321</td>
<td>2364</td>
<td>777</td>
<td>871</td>
<td>818</td>
<td>614</td>
<td>1444</td>
<td></td>
</tr>
<tr>
<td>Transferrin Saturation b</td>
<td>83</td>
<td>77</td>
<td>66</td>
<td>47</td>
<td>45</td>
<td>35</td>
<td>93</td>
<td>89</td>
<td>45</td>
<td>40</td>
<td>58</td>
<td>32</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>TIBC</td>
<td>276</td>
<td>287</td>
<td>271</td>
<td>244</td>
<td>344</td>
<td>238</td>
<td>389</td>
<td>319</td>
<td>345</td>
<td>362</td>
<td>313</td>
<td>329</td>
<td>329</td>
<td></td>
</tr>
<tr>
<td>Iron c</td>
<td>230</td>
<td>220</td>
<td>178</td>
<td>115</td>
<td>154</td>
<td>119</td>
<td>362</td>
<td>285</td>
<td>154</td>
<td>144</td>
<td>181</td>
<td>104</td>
<td>223</td>
<td></td>
</tr>
</tbody>
</table>

**Sex**
- Male
- Female

**Age**
- 18 yr
- 21 yr
- 13 yr
- 14 yr
- 4 yr
- 18 yr
- 7 yr

**Captive or Wild Born**
- Captive
- Wild

\( ^a \)Ferritin units = ng/ml.

\( ^b \)Transferrin saturation = %.

\( ^c \)Iron units = ug/dl.
ARE CHANGES IN ELEPHANT MIGRATION PATTERNS DRIVEN BY QUALITATIVE DIFFERENCES IN FORAGE?

Michael R. Loomis, DVM, MA, Dipl. ACZM
North Carolina Zoological Park

In 1980, a herd of approximately 250 elephants began migrating south out of Waza National Park, in northern Cameroon during the rainy season. Before 1980, the group usually stayed in WNP year round, feeding in Acacia woodlands in the dry season and on the floodplains during the rains. When an irrigation dam altered the Park’s hydrology, annual grasses replaced the floodplain’s perennial varieties, and the herd began migrating in the wet season. On-going satellite telemetry studies documented the emergence of a migration tradition, triggered by the rains, that sends the herd marching, nearly non-stop, 60 miles south to feed for several weeks at a specific site near Kaele. Then the herd invades millet fields leading to crop destruction, retaliatory poaching, chemical sprays and other harassments and occasional killing of nuisance elephants. The only deterrent that has had success is the use of pepper spray. A 1994 International Union for Conservation (IUCN) Reclamation Project re-established a small part of the plant community that used to feed the study-herd during the wet season. The returning native perennial grasses failed to reverse the southern migration and set the stage for this study to attempt to identify the nutritional factors underlying the herd’s use of the Kaele area. We hypothesize that the herd’s southern migration route is a function of quality differences between the food plants at Kaele and the food plants in WZP. We also hypothesize that understanding these differences will allow conservationists to improve the quality and increase the carrying capacity of protected areas and reduce future human/elephant conflicts by redirecting migrating elephants to low-conflict areas. Plant samples will be collected from WNP and the Kalea area and compared for nutritional value in an attempt to evaluate this hypothesis.
Kori bustards (*Ardeotis kori*) are reported to be omnivorous, consuming mostly insect and plant material in their grassland habitat. Nutrient content of the free-ranging diet has not been determined. The gastrointestinal tract of the kori is typical of an insectivorous bird. Currently, koris have been fed as "primarily carnivorous" omnivores, with whole vertebrate prey comprising large portions of the diet offered. It has been questioned whether the diets currently offered to captive koris are appropriate given their foraging behavior and gastrointestinal tract morphology. It is not clear whether the historic low breeding success is diet related, however when breeding has occurred, nearly 100% of all hand-reared chicks develop angel wing, a condition linked to high protein diets in waterfowl and cranes.

The food group comprising the greatest mean as fed proportion of kori diets in the US is whole prey and meat (68%). The importance of this contribution is evident when the nutrient content of these diets is compared to target nutrient values established for koris (Table 1). Target nutrient values (dry matter basis, DMB) were derived from several domestic and exotic avian species. Ranges for certain nutrients include a low end maintenance value and a high end breeding value. Some nutrients in the captive kori diets appear excessive, most evidently, crude protein. One of the approaches to decrease dietary protein may be to alter diets to include less whole prey and meat, in favor of increased dry nutritionally complete food (of moderate crude protein levels) and appropriate produce (leafy greens).

In 2003, consumption data was recorded for 2 hand-reared chicks was recorded by weight. One chick developed angel wing at day 6; the other chick did not develop angel wing. The chick that developed angel wing consumed a 33% protein diet between days 1-5, compared to 28% for the chick that did not develop angel wing. The growth rate of the chick that developed angel wing was 5.4% of body weight during that period, compared to 5.0% of body weight in the chick that did not. Growth rate of previously hand-reared chicks that developed angel wing ranged from 5.7-8.1% of body weight on a daily basis (mean = 6.8). Fast growth is important for production birds with significant muscle mass, but it is not the goal for captive exotic birds. Angel wing in waterfowl and cranes was successfully "treated" by reducing the crude protein content of the diet offered. For hand-reared koris it may be more appropriate to maintain dietary protein levels that allow normal growth in waterfowl and cranes (18-22% DMB).

Koris should be offered diets more appropriate for omnivorous birds, which more closely match target nutrient values. The protein content of diets offered to hand-reared chicks should fall within a range of 18-22%. When hand-rearing, diets should be recorded by weight of each individual food item. Growth rates should be restricted to less than 5% of body weight per day.
Literature Cited

Table 1. Mean nutrient composition of reported kori bustard diets (n=8) compared to target nutrient values (DMB).

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Range</th>
<th>Meana</th>
<th>SDb</th>
<th>Target Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein, %</td>
<td>29-56.1</td>
<td>40.4</td>
<td>9.0</td>
<td>16.5-26.4</td>
</tr>
<tr>
<td>Crude Fiber, %</td>
<td>1.0-7.0</td>
<td>3.8</td>
<td>2.0</td>
<td>-</td>
</tr>
<tr>
<td>Fat, %</td>
<td>9.7-22.6</td>
<td>15.6</td>
<td>5.0</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin A, IU/g</td>
<td>6.0-42.2</td>
<td>14.5</td>
<td>12.6</td>
<td>1.65-5.5</td>
</tr>
<tr>
<td>Vitamin D3, IU/g</td>
<td>0.6-2.5</td>
<td>1.4</td>
<td>0.7</td>
<td>0.22-1.2</td>
</tr>
<tr>
<td>Vitamin E, mg/kg</td>
<td>6.0-183.0</td>
<td>85.8</td>
<td>52.0</td>
<td>11.0-27.5</td>
</tr>
<tr>
<td>Thiamin, mg/kg</td>
<td>0.6-11.3</td>
<td>7.2</td>
<td>3.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Riboflavin, mg/kg</td>
<td>1.0-21.6</td>
<td>13.0</td>
<td>5.7</td>
<td>2.75-4.4</td>
</tr>
<tr>
<td>Niacin, mg/kg</td>
<td>5.8-239.0</td>
<td>128.9</td>
<td>61.5</td>
<td>22.0-71.5</td>
</tr>
<tr>
<td>Pyridoxine, mg/kg</td>
<td>0.6-13.3</td>
<td>8.7</td>
<td>3.6</td>
<td>3.3-5.0</td>
</tr>
<tr>
<td>Folacin, mg/kg</td>
<td>0.2-2.4</td>
<td>1.0</td>
<td>1.0</td>
<td>0.8-1.1</td>
</tr>
<tr>
<td>Vitamin B12, mg/kg</td>
<td>0.0-0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.003-0.01</td>
</tr>
<tr>
<td>Pant Acid, mg/kg</td>
<td>1.2-61.7</td>
<td>33.9</td>
<td>21.7</td>
<td>10.5-17.6</td>
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<tr>
<td>Choline, mg/kg</td>
<td>151.4-4536</td>
<td>2212.6</td>
<td>1321.8</td>
<td>990-1650</td>
</tr>
<tr>
<td>Biotin, mg/kg</td>
<td>0.0-0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>0.11-0.25</td>
</tr>
<tr>
<td>Calcium, %</td>
<td>1.0-3.0</td>
<td>1.6</td>
<td>0.6</td>
<td>0.66-2.75</td>
</tr>
<tr>
<td>Phosphorus, %</td>
<td>0.7-1.1</td>
<td>0.9</td>
<td>0.2</td>
<td>0.33-1</td>
</tr>
<tr>
<td>Magnesium, %</td>
<td>0.1-0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>0.05-0.055</td>
</tr>
<tr>
<td>Potassium, %</td>
<td>0.5-1.0</td>
<td>0.8</td>
<td>0.2</td>
<td>0.44-0.72</td>
</tr>
<tr>
<td>Sodium, %</td>
<td>0.2-0.5</td>
<td>0.3</td>
<td>0.1</td>
<td>0.13-0.18</td>
</tr>
<tr>
<td>Iron, mg/kg</td>
<td>145.2-294.4</td>
<td>223.5</td>
<td>50.6</td>
<td>55-77</td>
</tr>
<tr>
<td>Zinc, mg/kg</td>
<td>92.1-163.0</td>
<td>125.4</td>
<td>25.6</td>
<td>55-70.1</td>
</tr>
<tr>
<td>Copper, mg/kg</td>
<td>8.8-16.0</td>
<td>11.7</td>
<td>2.6</td>
<td>5.5-8.8</td>
</tr>
<tr>
<td>Manganese, mg/kg</td>
<td>12.9-137.5</td>
<td>71.8</td>
<td>39.3</td>
<td>66-72</td>
</tr>
<tr>
<td>Selenium, mg/kg</td>
<td>0.0-0.3</td>
<td>0.2</td>
<td>0.1</td>
<td>0.22</td>
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<tr>
<td>Iodine, mg/kg</td>
<td>0.1-1.3</td>
<td>0.7</td>
<td>0.4</td>
<td>0.33-0.44</td>
</tr>
</tbody>
</table>

a Mean and standard deviation (SD) based on 8 diets
b Target values based on NRC 1994 and Anderson 1995.
Cataracts of diverse etiologies occur commonly in many species and can often have serious consequences. The development of cataracts may be due to nutritional deficiencies or excess as well as hereditary factors, senile changes, metabolic or parasitic diseases, certain toxins or drugs, trauma, radiation therapy, or inflammation, among other etiologies. Whatever the inciting cause, cataracts result from the ensuing abnormalities in lens metabolism and subsequent disruption of lens fiber integrity.

Cataracts caused by nutritional excess or deficiency have been observed in several species. Nutritional toxins, such as mimosine from Leucaena leaves are known to cause cataracts in cattle. Cataracts in cultured fish are common worldwide, producing large economic losses, and have been reported with excesses in mineral supplementation as well as deficiencies in methionine, zinc, riboflavin, niacin, and vitamins A and C. In the early 1990’s, cats and dogs as well as timberwolves that were deprived of dam’s milk and fed a commercial milk replacer as neonates often developed perinuclear cataracts that were correlated with a deficiency of arginine among other amino acids. Additional amino acid deficiencies associated with cataracts include tryptophan, phenylalanine, valine, and histidine. One report suspected cataracts resulting from hypocalcemia, recognized as a metabolic cause of cataracts, in a kitten fed a pure meat diet. Marsupial pouch young fed cow’s milk can rapidly develop cataracts, and the proposed mechanism of this form of cataractogenesis, also demonstrated in rats, has recently been reviewed by Stanley. Briefly, the lactose in milk breaks down in the small intestine to glucose and galactose. Young marsupials are essentially galactose intolerant, having low levels of galactokinase and galactose-1-phosphate uridyl transferase activities needed for the breakdown of galactose. As galactose levels rise in the lens, galactokinase is overwhelmed, leading to metabolism of the excess galactose by the aldose reductase pathway to galactitol. The presence of galactitol changes the osmotic pressure, and water is drawn into the lens in a manner similar to that in the pathogenesis of diabetic cataracts. High levels of galactose in the lens can also lead to auto-oxidation, resulting in a significant increase in free radical formation, such as hydrogen peroxide and dicarbonyl compounds, which exceeds the normal antioxidant capacity of the lens and leads to further cataract formation.

Dietary antioxidants have long been correlated with the inhibition or delay of cataract formation. The reducing compound glutathione is found in high concentrations in the lens and acts to maintain lens transparency by detoxifying reactive oxygen species generated by ultraviolet light and metabolism, among other mechanisms. Several species have been shown to develop cataracts with nutritional deficiencies in antioxidants, including turkey embryos from hens fed a diet deficient in vitamin E as well as hunting dogs shown to have a deficiency in this vitamin.

Recent evidence from human geriatric studies has also supported the beneficial role of antioxidants in the diet. In the aging lens glutathione concentrations in the nucleus are low, leaving the lens more vulnerable to oxidative damage which leads to cataract formation. Many recent studies report strong correlations for vitamins C and E, as well as other dietary antioxidants, in diminishing the risk of cataracts in aging humans. The carotenoids lutein and zeaxanthin may also protect against the development of age-related cataracts and macular degeneration. Purified antioxidants, however, such as those in formulated diets, are not sufficient to account for their...
apparent protective effect against chronic disease; it is the complex mixture of antioxidants in whole fruits and vegetables and other foods that is likely providing a synergistic and additive protection.\textsuperscript{19}

\begin{itemize}
\item[19] Liu, RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutrition 2003; 78:517S-520S.
\end{itemize}
Sue Crissey developed the zoo animal nutrition matrix. This approach to offering animals optimal diets in captivity was presented in poster form at the 4th Conference on Zoo and Wildlife Nutrition in Orlando in 2001. Sue authored a paper providing details of the matrix that has been accepted for the special nutrition edition of the International Zoo Yearbook 2004. This abstract outlines the zoo animal nutrition matrix including its application to every day use.

The primary goal for zoo nutritionists is to provide optimal nutrition for animals in captivity. However, this task is complex. There are many factors to consider when formulating and evaluating exotic animal diets. The Zoo Animal Nutrition Matrix is a comprehensive approach to this challenging objective. The matrix consists of 4 categories: 1) consumption, 2) requirements, 3) health, and 4) management. Within these four categories are many subcategories that expand or contract to fit the needs of the situation. Figure 1 is an illustration of the matrix.

Factors affecting consumption of the diet are numerous. They range from food items including water, manufactured diets, invertebrates, supplements, produce, forage, browse, meat, and prey, to animal preference, form of the diet and quantity offered. Each of these factors have multiple concepts to consider. For example, form of the diet may encompass, live, frozen/thawed, hard, soft, as well as shelf life. The range of nutrient requirements for the evaluation in review may be affected by animal, season, life stage, stress, body temperature, known species requirements, feeding ecology, and morphology. These requirements may include as many as 64 nutrients and ultraviolet light. Health is multi-factorial including preventative measures, infectious agents, treatment, non-infectious agents, and dietary factors. Each of these factors in turn have multiple considerations as well. Those for dietary include deficiency and toxicity amount of nutrient and form. The final category is management which encompasses food storage, environment, enclosure and feeding regime. Within these areas factors such as temperature, stress, size of enclosure, type of enclosure, training, exercise, and behavioral enrichment are considered. It is probable that all considerations have not been explored within these 4 main categories of the matrix. Situations involving decision making for optimal nutrition have many intricacies and peculiarities that are unique and need to be individualized. Often it is assumed that once a diet is established that there is no need for further investigation. However, a change in any consideration in the matrix, effects several components. Consequently, offering optimal diets is a constantly evolving process. Therefore, a teamwork approach including nutritionists, veterinarians, animal managers, and keepers ensures all factors are considered in decision-making processes. Communication is the link between the components of the matrix and the professionals contributing to the team. For without communication no situation can be evaluated.

References:
RESEARCH AT A ZOO? IF SUE CAN DO IT, SO CAN YOU!

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Contributing to the limited database available on the nutritional needs of our captive exotic animals is an important function of a zoo nutritionist. A great diversity of projects encompass the challenge of zoo nutrition research from analyzing the nutrient content of diets commonly fed in zoos or factors affecting a wholesome diet to analyzing foods collected in the field consumed by free ranging animals. Trials defining a requirement for a particular nutrient, similar to domestic animal studies, in a zoo setting are difficult if not impossible to conduct. However, work can still be done to define parameters required to offer animals the most appropriate diets. In fact, research with captive exotic species can be rich indeed. Sue Crissey’s career at Brookfield Zoo provides examples of the opportunities that await. Projects have been divided into four categories 1) utilizing model species, 2) utilizing collection animals, 3) analyzing foods for nutrient content, 4) assessing health status with physiological sample collection and diet analysis, 5) collecting free-ranging animal data in the field.

Using a non-endangered species for an endangered species in a controlled study.

Hepatic iron accumulation in European starlings fed two levels of iron: Nestling and fledgling starlings were used a model for passerine species. The chicks were raised on an iron-controlled diet then assigned to either the controlled iron diet or a high iron diet. Birds were euthanized at baseline, 8 wks and 16 wks. Body, liver and spleen weights were measured and iron and copper concentrations were determined. Iron accumulated on both diets, but was slower with the controlled iron diet.

Utilizing the animal collection.

Use of raw meat-based or dry kibble diets for sandcats: Eight sandcats were used to test the digestibility of a raw meat-based diet and a dry kibble diet. Dry matter, crude protein and energy intakes and digestibilities were evaluated. The kibble diet had lower digestibility for the nutrients examined compared to the raw diet. Blood concentrations of taurine, vitamin A, retinyl palmitate, beta-carotene, calcium and phosphorus were determined. Concentrations varied depending on the diet fed. The study concluded that diets formulated for small captive exotic felids should be evaluated with respect to diet type and nutrient utilization.

Vitamin D and kidney function in Callimico: The purpose of the study was to examine vitamin D status and if possible link it with indices of renal function. Blood samples were collected and analyzed for blood urea nitrogen(BUN):creatinine ratio, BUN, creatinine, hemoglobin, hematocrit, sodium, citric acid, calcium, phosphorus, bilirubin, albumin, alkaline phosphatase, chloride, 1,25 (OH)₂ D and 25(OH) D. Three primary results were noted 1) Brookfield Zoo population had kidney dysfunction at some level, especially the males, 2) vitamin D metabolites normally found in goeldis monkeys were lower than other New World monkeys 3)although dietary vitamin D did not cause renal problems, the renal dysfunction may have influenced the vitamin D metabolites.
Food Composition:

**Cholesterol, fat and fatty acid concentrations in whole mice fed to captive exotic animals:** This project examined the concentrations of dry matter, cholesterol, saturated fat, mono-unsaturated fat and fatty acids in four sizes of mice. Dry matter increased with age/size of mice, while cholesterol levels decreased. Significant differences were found among mice categories with capric, lauric and myristic acids. Generally levels of fatty acids were high and fell between those of beef and fish. Mice contained higher levels of polyunsaturated fatty acids and lower levels of saturated fatty acids than beef. The possibility of dietary excess exists along with potential eye and tissue abnormalities.

**Irradiated diets: microbial destruction, consumption and fecal consistency:** Two frozen, raw horsemeat-based diets fed to captive felids at Brookfield Zoo were irradiated to determine the extent of microbial destruction and whether irradiation would affect consumption and/or fecal consistency. Fifteen cats representing seven species were fed both the irradiated and non-irradiated diets. No differences in either consumption or fecal quality were noted. Irradiation of the meat diets did result in microbial destruction, however storage time between irradiation and sampling may affect microbial reduction. Irradiation would, however be an appropriate method for reducing potentially pathogenic bacteria.

Assessment

**Databank Project:** Blood nutritional parameters of primates and carnivores at four zoos (Brookfield, Lincoln Park, Ft Worth and North Carolina Zoos) were examined. Blood from healthy animals were obtained for analysis of minerals, serum chemistries, vitamins A, D, E and D carotenoids, lipids and amino acids. These data provide a baseline for understanding the health and nutritional needs of captive animals. This project continues with piscivorous birds.

**Cardiovascular disease risk:** Gorillas appear to have a high risk for cardiovascular disease given published lipid profiles but unlike humans, do not commonly exhibit atherosclerosis or succumb to myocardial infarction. The hypothesis is that a full risk assessment (not just limited to lipid profile) will show distinct differences in risk patterns between humans and gorillas. The biochemical markers examined includes: cholesterol, triglycerides, direct measured high density lipoprotein and calculated or directly measured low density lipoprotein, plasma homocysteine, lipoprotein (a) and high sensitivity C-reactive protein. The incidence of cardiovascular disease occurrence also will be compiled and compared. Diet information will be collected by survey and the estimated nutritional composition of the diet for each species at each institution will be compared with nutrient requirements and human heart-healthy diets.

Field Work

**Nutritional status of free-ranging howler monkeys in Veracruz, Mexico:** The nutritional status of six Mexican mantled howler monkeys was examined using biochemical indices. Blood samples were analyzed for minerals, serum chemistries, vitamins A, D, E and carotenoids and lipids. Plant samples of foods consumed by the monkeys were collected. Serum chemistries were somewhat different than published values. Concentrations of other nutrients were similar to those published for captive and wild primates. The most significant result was the detection of cadmium, an environmental contaminant, in the blood of the howlers. It was not found in the plant samples. This has implications for human and animals living in the area.
ORAL ADMINISTRATION OF PHARMACEUTICALS IN AQUARIA

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Oral administration of pharmaceuticals is relatively non-invasive means, considering the capture and restraint necessary for parenteral routes of administration of medications. Oral administration of pharmaceuticals is also relatively inexpensive, considering the cost for professional staff and equipment involved in capture and restraint. Needs of the individual, physiochemical properties of the drug, type of medicated feed, and environmental parameters must all be considered. Fishes, mammals, and birds require different considerations in administering oral medications.

There are several points to consider before administering oral medications in fishes. Oral administration is often preferable to catching numerous individuals in a larger aquarium. Depth of the exhibit, contours, and décor can also be factors supporting a decision to opt for oral medications. However, animals may be off feed, and medications can further impact anorexia by decreasing palatability of the feed. Unaffected animals will be inadvertently dosed, and the sediment and microflora can be affected by antibiotics.

Other considerations for medicated feed must be the diet specifications of the species (carnivorous, herbivorous, and omnivorous). Palatability can be improved by addition of certain amino acids such as glycine and alanine. Stability of the feed and its nutritive components should not be neglected. Of particular importance are the presence of thiaminase (degradation of Vitamin B-1) and the potential for oxidative damage to fatty acids and subsequent depletion of Vitamin E. The feed should be stable in the aquatic environment, and medications should also be stable. For example, bioavailability of fluoroquinolone and tetracycline antibiotics will be decreased in seawater due to chelation of divalent cations (Mg²⁺, Ca²⁺). pH partitioning effects can also occur with antibiotics such as aminoglycosides. Finally, size and texture of the feed should be appropriate for the species.

Several options for medicating feed are available for fish. These include gelatin prepared diets, Artemia soaks, commercial and homemade pellet feed, and whole fish stuffed with medications. Gelatin diets are particularly important when administering quarantine protocol medications (praziquantel, fenbendazole, and metronidazole) to fish. The quarantine protocol can also incorporate bath treatments (formalin, chloroquine, copper). These protocols must take into account safety and efficacy in the species treated, as well as the parasites or other disease processes discovered. It is a good idea to get fish accustomed to unmedicated gelatin diets, or those fortified with vitamins and minerals, should medicated gelatin diets become necessary at a later date.
Considerations for oral administration of medications in marine mammals and birds are similar to those of fish with some exceptions. If animals are already eating, fish stuffed with medications, vitamins, and minerals can be offered. Draining a pool to give injections requires great expense in water and professional labor costs. However, pool drains and other techniques for capture may still be necessary if an animal is not eating. Force-feeding often involved prying open the animal’s mouth and hand-assist feeding in the mouth or throat. Extreme caution should be exercised to avoid injury or infection from oral microflora.

A palatable and stable diet tailored to the species and individual’s needs is crucial. Neonates require artificial milk formulas. Stranded or anorectic animals can be fed a herring gruel (890 – 2300 cal/kg) fortified with fish oils. Manatees require a vegetarian gruel of monkey chow biscuits, lettuce, and other vegetables. If whole fish stuffed with medications are to be fed, then the animal should be tube fed fresh water to rehydrate the gastrointestinal tract. Regurgitation or inadequate digestions are risks in marine mammals and birds. Parenteral nutrition, medication, and rehydration are an option, albeit a more expensive and labor intensive one.
THE EFFECTS OF COLD STRESS SYNDROME AND DIET ON THE GUT MICROFLORA AND LONG TERM SURVIVABILITY OF THE FLORIDA MANATEE, TRICHECHUS MANATUS LATOROSTRIS

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Manatee cold stress syndrome is a cascade of events that predisposes manatees to opportunistic pathogens involving multiple organ systems. Affected manatees are forced to make dietary changes, which alter their gut flora. Manatees depend on gut microbes for digestion. There is little known about the complex nature of events that cause cold stress syndrome and even less on how microbial populations in the gut may be affected, which makes it difficult to ascertain the damage caused by starvation and hypothermia, and thus management decisions to ensure the humane treatment and conservation of the endangered Florida manatee become challenging. The objectives of this study were to establish microbial populations, obtained from the manatee gut, using in vitro batch culture techniques, and determine the effects of fasting on microbial fermentation and the ability of microbes to recover from experimentally induced cold stress syndrome.

The manatee processes food similar to terrestrial hindgut fermentors, however there are some anatomical differences. The manatee’s GI tract has several specialized areas including the accessory digestive gland (cardiac gland), duodenal diverticulae, duodenal ampulla, cecum with cecal horns, and large intestine, which contribute to the manatee's ability to be highly efficient in digesting its diet (80-95%) (Burn, 1986). The nature of digestion (cellulose breakdown) and preliminary measurements of the gut pH suggests that microbial fermentation is likely present in a large portion of the manatee GI tract including the duodenum, cecum, and large intestine.

Since the Florida manatee is considered a hindgut fermentor, the general knowledge of domestic terrestrial herbivorous animals’ GI anatomy, physiology, and microbial ecology, and the techniques used to culture gut contents of herbivorous animals can be employed to closely mimic the manatee microbial ecosystem. Between June and July 2003, manatees that perished were transported to the Marine Mammal Pathobiology Laboratory (St. Petersburg, FL) for necropsy within 24 hours and were used to obtain gastrointestinal microflora that were subsequently incubated in vitro for the fermentation study.

During each necropsy, the entire GI tract from the stomach to the rectum was removed from the animal. In order to minimize contamination of GI contents from other sampling sites within close proximity, a tightly tied string was used to immediately
isolate sampling areas. The sampling protocol followed for this study was similar, with a few modifications, to the one used in a previous study for rumen microbial in vitro fermentation studies (Lynch and Martin, 2002). Duodenal and proximal, middle, and distal colon contents were obtained and squeezed through four layers of cheesecloth into a 1000 mL Erlenmeyer flask with an O₂-free CO₂ headspace. The flask was then placed in a 39°C water bath and remained undisturbed for 30 min, allowing the feed particles of digesta to rise to the top. Particle-free fluid from the flask were anaerobically transferred (20% vol/vol) to a medium (pH 6.5) containing 292 mg of K₂HPO₄, 240 mg of KH₂PO₄, 480 mg of (NH₄)₂SO₄, 480 mg of NaCl, 100 mg of MgSO₄·7H₂O, 64 mg of CaCl₂·2H₂O, 4,000 mg of Na₂CO₃, and 600 mg of cysteine hydrochloride per liter. Particle-free fluid and medium was mixed, and 40 mL were transferred anaerobically to 160-mL serum bottles containing either no substrate, or 0.4 g of freeze-dried sea grass (Thalassia testudinum). The previously prepared bottles were sealed (CO₂ atmosphere) with butyl rubber stoppers and aluminum caps to contain gas pressure, and after inoculation were placed in a 39°C waterbath for either 24 h or 48 h and periodically mixed. At 12h (24 h incubation) and 24 h (48 h incubation), 0.4 g of soluble sea grass (Thalassia testudinum) were added to half of the serum bottles. After 24 h or 48 h of incubation, the serum bottles were removed from the water bath and refrigerated to inhibit fermentation. The bottles were transported in dry ice to North Carolina State University for subsequent analyses. Another experiment, using the same protocol as described above was performed using contents obtained from a cannulated non-lactating dairy cow fed a predominantly forage diet. Methane, culture pH, and volatile short chain fatty acids (SCFA) (mM and molar %) were determined for both manatee & rumen fermentations. Data were analyzed using the PROC GLM procedure of SAS and significance was reported at \( P < 0.05 \).

Incubation of mixed manatee microbial cultures based on in vitro fermentation techniques used for rumen contents (Lynch and Martin, 2002) proved successful. Overall seagrass incubated with manatee cultures produced significantly higher concentration of total SCFA, lower concentrations of methane and resulted in a lower pH than rumen fluid fed the same substrate. Microbial cultures from the manatee gut also resulted in a higher concentration of acetate when compared to rumen microbes fed the same substrate. A higher acetate, lower methane fermentation pattern suggests that manatee microflora were more efficient in reducing the potential loss of carbon energy in the form of methane. Delaying feeding for 24 hr resulted in significant reductions in overall fermentation, however fermentation was not completely inhibited as we had expected. Our results indicate that starving the microbes for a period of 24 h did not result in the cessation of microbial growth. It is possible that the effect of starvation on microbial growth may be more pronounced at intervals greater than 24 or 48 h.

References:

Identification of prey in wild carnivore feces provides useful data on dietary habits and predator-prey interactions. Traditional prey detection methods in stool rely on gross or microscopic inspection for distinctive hair, feather, or bone which has limited sensitivity and specificity. Molecular techniques have successfully identified fecal origins in the field presumably by amplifying DNA from sloughed colonic cells of the host species. Expansion of these techniques could potentially provide a highly sensitive and specific means of identifying consumed prey in carnivore scat. In this investigation, we tested species-specific primers targeting various-sized portions of the cytochrome-b (cyt-b) gene encoded on mitochondrial DNA (mtDNA) to distinguish between host species and consumed prey species. Our carnivore model was the red wolf (Canis rufus) and chicken (Gallus gallus) and white-tailed deer (Odocoileus virginianus) were model prey species.

The experiment was divided into 3 phases. First, species-specific primers were tested independently on pure tissue extracts for cross-annealing using standard PCR techniques to ensure primer fidelity. Sequencing of amplification products is currently pending. Results will be BLASTed in the GenBank database to confirm the expected target sequence for each species. Second, wolf fecal samples were then spiked with serial dilutions of prey tissue, extracted, and PCR was conducted to determine the assay sensitivity. Multiplex PCR was attempted to improve assay efficiency. Third, wolves were fed prey meat and their scat was examined grossly for bone or tissue fragments in addition to performing molecular assay for prey detection.

Results showed cyt-b can be used to distinguish among the 3 species. Assay sensitivity was interpretable only for deer and detected between 80–300 ng deer/1 mg spiked feces. Multiplex PCR was unreliable for detecting both host and prey cyt-b in a single reaction tube. Prey mtDNA could be recovered and amplified from fresh scat up to 36 hours after a single feeding. Preliminary results of this fecal molecular assay appear promising for identifying prey in carnivore scat. More work needs to be done to optimize the assay for multiplex PCR and to test the duration of prey detection following the consumption of various species in both open and blinded food trials.

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ATTEMPTED THERAPY OF SUBCUTANEOUS LIPOMAS IN BUDGERIGARS (MELOPSITTACUS UNDULATUS) USING DIET MODIFICATION AND L-CARNITINE SUPPLEMENTATION

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Introduction

The occurrence of subcutaneous lipomas is frequently reported in psittacine birds. A number of species seem to be particularly predisposed to developing these benign tumors including Amazon parrots (Amazona sp), rose breasted cockatoos (Eolophus roseicapillus) and the budgerigar (Melopsittacus undulatus). Development of lipomas in psittacine birds has been associated with dietary induced obesity, hypothyroidism and genetic factors. The association with obesity appears to be strong, as anecdotally lipomas occur more frequently in over-conditioned birds. Different therapeutic interventions have been attempted with varying degrees of success. These include surgical removal, dietary modification, and oral administration of thyroxine. None of these approaches have been scientifically evaluated.

L-carnitine is an amino acid derivative that is involved in the transport of long chain fatty acids across the mitochondrial membrane for subsequent oxidation. Diet supplementation with L-carnitine may modify body condition and composition, and has been tried in both birds and mammals with varying results. Supplementation for this purpose in birds has been evaluated in poultry and pigeons. L-carnitine supplementation did not affect carcass characteristic in chickens, but did significantly lower blood triglyceride and fatty acid levels. In pigeons, supplementation was associated with an increased body weight in parent birds and an accelerated growth rate in their offspring. L-carnitine supplementation also appears to promote antibody production in chickens through unknown mechanisms.

Theoretically, lipoma size should decrease in tandem with other depots of adipose tissue. Therefore, decreasing the energy consumption, increasing exercise, or reducing fat in the diet should help reduce lipoma size. Manipulating fat metabolism with L-thyroxine or dietary supplements such as L-carnitine may also help. The purpose of this study was to evaluate the effect of dietary modification and L-carnitine supplementation on bodyweight and lipoma size in a group of adult budgerigars.

Summary of Research Project

A flock of thirty-two adult male and female budgerigars (Melopsitticus undulatus) was used for this study. Seventeen of the 32 birds had detectable subcutaneous lipomas at the beginning of the study. Fifteen birds without lipomas served as controls. The lipomatous and control birds were randomly assigned to one of three different dietary groups. The first dietary group continued to receive the seed diet fed prior to starting the study. The remaining groups were fed either a commercially available pelleted diet (Roudybush low-fat maintenance, Roudybush, Inc., Paso Robles, CA, USA), or the same pelleted diet supplemented with approximately 1000 mg/kg L-carnitine (Lonzia Inc., Fairlawn, NJ). Body weight and cervical lipoma measurements were recorded prior to beginning the study and then at approximately 2-4
week intervals for a total of seven measurement episodes during the 102 day observation period. Histologic examination of the lipomatous masses was performed on 15/17 budgies with tumors.

**Results**

All the masses histologically examined were confirmed as lipomas. There was an increase in body weight in both the lipomatous and control budgerigars receiving the 100% seed diet. Lipoma size increased in the groups receiving 100% seed and unsupplemented pellets. Average lipoma size decreased in the lipomatous budgerigars receiving the L-carnitine supplemented pelleted diet. Four control birds (three receiving 100% seed and one receiving L-carnitine supplemented pellets) developed cervical lipomas during the course of the study. These data shows that feeding a 100% seed diet to budgerigars can result in inappropriate weight gain and that L-carnitine supplementation may be useful in management of lipomas in budgerigars.

**References**


*Adapted from:

DIETARY AND HUSBANDRY STRATEGIES TO DECREASE THE RISK OF TOXOPLASMOSIS IN PALLAS’ CATS

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Pallas’ cats (Otocolobus manul) are small felids indigenous to central Asia that are threatened with extinction. In the mid-1990’s, 24 wild-born Pallas’ cats were imported from Russia and Mongolia as founders to establish a captive population in North American zoos under the guidance of a Species Survival Plan (SSP). Between 1996 and 2001, 65 kittens were born in 17 litters, but nearly 60% of these kittens died within 4 months, primarily due to toxoplasmosis.1,2 Mortalities in Pallas’ cat kittens from the protozoan, Toxoplasma gondii, occur at birth or within the first few weeks, suggesting in utero transmission, and at weaning, implicating lactogenic transmission or consumption of cysts in conventional raw meat diets. Sporadic mortalities also occur in adults where there is circumstantial evidence of reactivation of T. gondii during stressful events like shipping, pregnancy, and nursing.2

In 2000, 4.2 Pallas’ cats, IgG positive for T. gondii, were donated to North Carolina State University College of Veterinary Medicine (NCSU CVM) to establish a research colony. One goal of the colony was to produce specific pathogen free (SPF) Pallas’ cat kittens for the SSP. The adult cats were housed in a secure indoor facility to eliminate the possibility of ingesting wild rodents and birds, which could re-infect the cats with a different strain of T. gondii. Fluorescent lights were timed to mimic seasonal photoperiods so that the breeding season (January-March) would begin with increasing daylight. The commercial frozen raw horse meat diet was switched to a different brand, Milliken Feline Diet (Scarborough, Ontario M1V 3F1, Canada), and only gnotobiotic rodents were offered for enrichment.

Three of six adult Pallas’ cats had 6-fold increases and one had a 4-fold increase in their T. gondii titers after their arrival at NCSU, suggesting a reactivation of their previous infection. One female with a titer of 1:16,384 died acutely from a severe meningo-encephalitis caused by T. gondii 4 months after arrival and 3 days after a physical examination. Within 8 months, all cats with elevated titers had returned to their pre-shipment titers and no cat has experienced any increases in over a year.

In 2002, six kittens were born to a female that arrived in 2001 with an IgG titer of 1:64 to T. gondii. The kittens were pulled at birth and cross-fostered onto nursing, SPF domestic cat queens. They were weaned onto IAMS Kitten and remain on IAMS as adults. They are seronegative for T. gondii and are currently part of the SSP. The IAMS feline diet appears to support growth and reproduction in these cats. An unrelated male and female born in 2002
produced healthy kittens in 2003. IAMS is currently funding a study to determine if there are any significant differences in nutritional status/health/reproductive parameters of Pallas' cats maintained on IAMS feline diet compared to a commercial raw horse meat diet.

Husbandry and diet changes appear to have minimized the risk of clinical toxoplasmosis in the NCSU Pallas' cat colony. The relative contributions of each are impossible to assign but are probably complementary. Pulling kittens within 6 hours of birth would not eliminate in utero transmission or lactogenic transmission necessarily. The queen, however, was seronegative for *T. gondii* during pregnancy and 10 weeks post-partum despite a positive titer one month prior to the breeding season. The implications are that off-exhibit facilities might be appropriate when breeding Pallas' cats. Whether the female's titers would have increased if she had raised the kittens is entirely speculative. What is clear is the need to prevent the introduction of *T. gondii* into the Pallas' cat SSP. Indoor exhibits and processed commercial diets are two simple management strategies for reducing this infection in a highly susceptible species.

**Literature cited:**
