NC STATE UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

Sixth Crissey Zoological Nutrition Symposium

Raleigh, North Carolina

December 10 and 11, 2010

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

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

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

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

""I don't know that I was a great teacher, but in almost everything I did, I tried to encourage others to look for opportunities to be helpful to people and to appreciate our natural world."
Sue Crissey 2002

Susan D. Crissey

Sixth Crissey Zoological Nutrition Symposium
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Friday, December 10, 2010

			Page
12:30- 1:00 pm	Registration		
1:00 – 1:30 pm	Conference Introduction and Welcome	Michael Stoskopf Kimberly Ange-van Heugten Michael Power	

Session I: Nutrition and Conception and Gestation
Chair: Barbara Wolfe, The Wilds

1:30 – 1:45 pm	Obesity, Insulin Resistance and Abnormal Reproduction in the Domestic Horse as Model for Perissodactyls in Zoological Institutions?	Vick, Dennis	1
1:45- 2:00 pm	The effects of Energy Balance on Reproduction in the Common Marmoset (*Callithrix jacchus*)	Power, Tardiff	3
2:00 – 2:15 pm	Serum Adiposity Parameters and Reproductive Problems in Female White Rhinos	Long et al	7
2:15 – 2:30 pm	Question & Answer Session		
2:30 – 2:35 pm	Present Conundrum I - Contraception Drugs or Hormones in Feed	van Heugten	9
2:35 – 3:15 pm	**Break and First Poster Session**	**Blue Commons**	
3:15 – 3:30 pm	Discussion of Conundrum I	van Heugten	
3:30 – 3:45 pm	Development of Obesity Begins at an Early Age in Captive Common Marmosets (*Callithrix jaccus*)	Power et al	11
3:45 – 4:00 pm	Nutritional Causes of Common Giraffe Health Issues and How They're Related	Wolfe, Clauss	15
4:00 – 4:15 pm	North Carolina Zoo Gorilla Chow-Free Diet: A Keeper's Update	Jesue	13
4:15 – 4:30 pm	Preliminary Behavior and Health Effects of a Biscuit-Free Diet in Captive Gorillas	Hoelbein-Less et al.	17
4:30 – 4:45 pm	Question and Answer Session		
4:45- 4:50 pm	Presentation of Conundrum II – Do Plastics Used in Feeding and Storage Pose a Reproductive Risk?	Michael Stoskopf	19

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5:00 to 6:30 pm **Evening Reception with Light Appetizers** **Green Commons**

6:30 to 7:30 pm **Key Note Address**

The Origin of Lactation and the Evolution of Lactation Strategies 23
by
Olav Oftedal, PhD

Saturday, December 11, 2010

8:00 – 9:00 am Continental Breakfast

Session II - Nutrition and Lactation
Chair: Olav Oftedal, Smithsonian

9:00- 9:15 am	Discussion of Conundrum II	Stoskopf	
9:15- 9:45 am	Geometry and the Nature of Nutrition	Raubenheimer	25
9:45–10:00 am	Dietary Practices for Hand-Rearing of Infant Chimpanzee (*Pan troglodytes*) at the North Carolina Zoo	Web, Ireland	27
10:00–10:15 am	Proximate Composition of the Milk of the Owl Monkey	Behler et al.	29
10:15–10:30 am	Hand-rearing Neonatal Eastern Cottontails- A Survey of Methods Used for Success	Buckanoff	33
10:30–10:45 am	Question and answer Session		
10:45–10:50 am	Presentation of Conundrum III - Can Contraception Affect Nutritional Husbandry?	McComb	35

10:50–11:15 am **Break and Second Poster Session**

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Saturday, December 11, 2010 (continued)

Session III - General Nutrition I
Chair: Eric van Heugten - NCSU

Session IV - General Nutrition II
Chair - Michael Power - Smithsonian

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Saturday, December 11, 2010 (continued)

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Raleigh, North Carolina

POSTERS

OBESITY, INSULIN RESISTANCE AND ABNORMAL REPRODUCTION IN THE DOMESTIC HORSE AS MODEL FOR PERRISODACTYLS IN ZOOLOGICAL INSTITUTIONS?

Mandi M. Vick and Patricia M. Dennis, DVM, PhD, DACZM
Cleveland Metroparks Zoo, Conservation & Science, 4200 Wildlife Way, Cleveland, OH 44109

High body condition is associated with inflammation and insulin resistance in the domestic horse, characterized by increased serum concentrations of the hormones leptin and insulin as well as increases in pro-inflammatory cytokines. Obesity and insulin resistance in the horse have also been linked to both abnormal reproductive function and other disease syndromes such as laminitis. In relation to reproductive function, obese mares can experience repeated failure of ovulation and the development of luteinized follicles. These luteinized follicles produce progesterone comparable to luteal concentrations, are resistant to treatment with prostaglandin and can persist for periods of up to 3 months, impeding conception and reproductive success. Other members of the order Perissodactyla including endangered equids and rhinoceros maintained in zoological institutions may exhibit increased body condition related to increased caloric intake and decreased activity in the *ex situ* environment. Anovulatory follicles have also been reported in the Przewalski's horse, white rhinoceros and black rhinoceros in zoos. However, it is unclear whether body condition and insulin resistance may be a contributing factor. There are no data on physiological measures of body condition, inflammation, or insulin sensitivity in these species. Preliminary results from studies currently underway suggest that black rhinos in zoos exhibit higher concentrations of inflammatory cytokines, insulin, and insulin to glucose ratio than free-ranging counter parts. If conclusive evidence of increased body condition and insulin resistance is demonstrated, future studies examining the relationship between body condition, follicular dynamics and ovulation in Perissodactyls are warranted.

THE EFFECTS OF ENERGY BALANCE ON REPRODUCTION IN THE COMMON MARMOSET (*CALLITHRIX JACCHUS*).

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[2] Barshop Institute for Longevity & Aging Studies, University Of Texas Health Science Center, San Antonio TX 78245

The common marmoset (*Callithrix jacchus*) is a small (ca. 350g adult weight) New World monkey. Originally from the northeast region of Brazil, common marmosets have spread throughout the Brazilian coast, largely due to human transport. Common marmosets can now be found in parks as far south as Sao Paolo, and on farms in much of the countryside. The common marmoset has one of the highest rates of reproduction among anthropoid primates, routinely producing twins and experiencing a fertile post partum estrus. Reproduction in the common marmoset has no inherent seasonal rhythm; in farms near the Poço das Antas biological reserve in the state of Rio de Janeiro, common marmoset females were found to be pregnant in any month, in contrast to the native related species, the golden lion tamarin (*Leontopithecus rosalia*), which has distinct major and minor birth seasons. Common marmosets represent a "weedy" primate species, able to rapidly colonize new areas. Their reproductive patterns are more variable than is normal for most primates, which makes them an interesting species within which to investigate the regulation of reproductive output.

In captive marmosets, interbirth interval, ovulation number, litter size, birth weight, milk composition, and infant growth all show significant variation, and much of the variation appears to be associated with maternal body weight and body weight change. For example, marmosets routinely ovulate twice each cycle. Four of five females that were placed on a high energy-density diet gained weight; those four females ovulated three eggs during their next cycle. The lone female that did not gain weight ovulated two eggs. The results presented here concern the effects of energy balance, as measured by weight change, on gestation. The gestation results come from energy restriction experiments.

Methods

Expected ad lib consumption was estimated from the results of a series of 2-day feeding trials on pregnant females in this population using this diet, as well as published estimates of energy intake in pregnant marmoset females (Nievergelt & Martin, 1999). The relative intake during pregnancy was similar between the two colonies, averaging 148 kcal/kg body weight in our colony and 158 kcal/kg in the Nievergelt & Martin study. Restricted females were provided with 75% of estimated ad lib energy intake (111 kcal/kg body weight per day). They were fed a 25% protein diet, resulting in their protein consumption being roughly equivalent to that of ad lib fed females who were fed a 15% protein diet. The diets were formulated such that all other nutrients in the diet (e.g., calcium, iron, folic acid, vitamins B_6 and B_{12}) were in sufficient excess that a 75% restriction would still provide adequate nutrition. It was not necessary to measure maternal food consumption in the restricted groups, as all females consumed all of the diet presented to them on each day of restriction. Control of food intake was accomplished by separating females on one side of the double-unit home cage. Females had visual, olfactory, auditory and limited tactile access to the other members of their family group through a mesh door dividing the two units. A control group of pregnant females (n=5) was fed in the same fashion as restricted females, but given ad lib access to food, to determine whether the feeding separation, in and of itself, had an effect on maternal weight gain and pregnancy outcome.

All females were given an ultrasound exam every two weeks during each pregnancy to determine stage of pregnancy and size and condition of embryos/fetuses. Maternal weights for all pregnant females in the colony are measured, on average, once a week, using the methods described in (Tardif et al., 2001). Restricted females were, on average, weighed twice a week. Infants were weighed at approximately 36 hours following delivery, and then approximately once per week until weaning (about 70 days post partum).

Results

During the period of pregnancy encompassing early-mid restriction (day 60-100), control females had a median weight gain of 0.80 grams per day while early-mid restricted females had a median weight gain of 0.0 grams. 74.3% of control females gained weight during this period and none lost weight. In contrast, no early-mid restricted females gained weight and 3 of 7 had a linear weight loss.

Energy restrictions in both early-mid and mid-late pregnancy had significant effects on length of pregnancy ($F= 32.14$, $df = 3$, $p < 0.0001$). Early-mid restriction reliably induced loss of pregnancy prior to term. Pregnancy loss occurred at an estimated 11 to 47 days following initiation of restriction (Mean 25 +/- 11.08 s.d.). The average estimated gestational age of pregnancy loss was 92 days. Of the mid-late restricted pregnancies, 4 of 7 were normal term length while three were preterm deliveries, at 101, 117 and 132 days. There were no pregnancy losses from 60-130 days for the control pregnancies.

There was no relation between pre-pregnant weight, daily weight change and estimated delivery age among early-mid restriction pregnancies, however, there was a significant relation between these variables in mid-late restricted females. Higher daily weight loss was associated with earlier delivery age (Spearman's rho = 0.889, $p<0.007$; see Figure 1). Smaller females tended to have higher weight losses (Spearman's rho = 0.704, $p=0.077$).

Discussion

The effects on pregnancy outcome of a relatively modest energy restriction suggest that the common marmoset is particularly sensitive to restriction during early-mid gestation, a period during which the placenta is undergoing exponential growth. All pregnancies restricted during this time period were lost significantly prior to term and none resulted in live deliveries. In contrast, there were no pregnancy losses prior to term when females were fed ad lib, either separately or in their social group. The complete lack of any pregnancy losses between days 60 and 130 post partum in the control group indicates that neither separation of dams for feeding nor routine handling for ultrasonography interfered with normal gestation.

For the energy restriction later in gestation, dams that lost weight faster had earlier delivery ages and dams that lost weight faster tended to have lower pre-pregnant weights. This result suggests that, unlike in the early-mid gestation restriction, larger females during this time period were perhaps protected from restriction-induced weight loss and, therefore, from risk of pre-term delivery.

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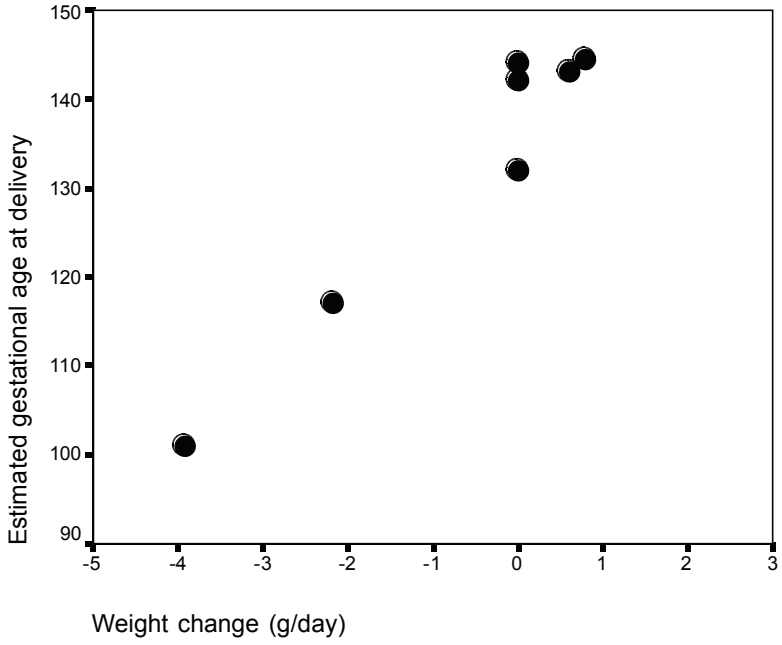
Figure 1. There was a linear relationship between maternal weight change and gestational age at delivery for females restricted from mid-to-late gestation. Term = 143 days.



SERUM ADIPOSITY PARAMETERS AND REPRODUCTIVE PROBLEMS IN FEMALE WHITE RHINOS

Lindsey J. Long, DVM, Dipl. A.C.V.P.M.[1], Barbara A. Wolfe, DVM, Ph.D, Dipl. A.C.Z.M.[2], and Patricia M. Dennis, DVM, Ph.D., Dipl. A.C.Z.M.[1,3]

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In 2004, the AZA Rhinoceros Research Advisory Group generated a list of priorities that should be examined in our captive rhinoceros population. Two of the stated priorities are: factors involved in reproductive failure of the Southern white rhino F_1 population and the etiology of reproductive pathologies in all rhino species. We hypothesis that obesity in the captive population as well as problems related to insulin/glucose metabolism are factors involved in reproductive pathologies of the Southern white rhino F_1 generation.

Obesity is a risk factor associated with numerous disease pathologies in multiple animal species and is a known problem in many captive animal populations including rhinos.[1] Concerning reproductive problems, obesity is a known risk factor for polycystic ovary syndrome, erratic estrous cycles and leiomyomas in humans.[2-4] Obese mares exhibit an extended interval between successive ovulations and prolonged periods of elevated circulating concentrations of progesterone.[5] Besides being a biomarker for adiposity, leptin plays a role in reproductive activity at the level of the hypothalamic-pituitary axis, regulation of early embryo cleavage and development, and has an inhibitory effect on developing ovarian follicles in humans and other animals.[4-11] Adiponectin, which decreases in obesity, is negatively associated with plasma insulin and may be involved in insulin resistance, hyperandrogenism, and polycystic ovary syndrome in obese humans.[4,10]

Our objective is to compare biomarkers of adiposity (leptin, adiponectin, IGF-I, and insulin:glucose) from the captive female white rhino population to reference values generated from wild female white rhinos to determine if the populations are similar. Evaluation of the captive white rhino population will be conducted further by examining female white rhinos that have had reproductive pathologies confirmed or which have been unsuccessful in reproducing to females who have shown repeated success or which have been evaluated and exhibit no reproductive pathologies.

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SIXTH CRISSEY ZOOLOGICAL NUTRITION SYMPOSIUM

CONUNDRUM I

Contraception Drugs or Hormones in Feed

Eric van Heugten

Successful management of captive animal populations at zoological institutions includes comprehensive breeding programs. These programs may be designed to expand animal collections and conserve endangered species. On the other hand, breeding programs may focus on controlling population growth to maintain appropriate numbers of animals that can be managed within space and financial constraints, to prevent inbreeding and maintain genetic diversity of collections. Contraception is a common and effective method to manage reproduction in animals. Thus, contraceptives can be administered to allow breeding of certain animals, but prevent pregnancy in others.
What are the pros and cons of offering contraception drugs in the feed (pre-milled, sprayed, soaked in, etc)?

THE DEVELOPMENT OF OBESITY BEGINS AT AN EARLY AGE IN CAPTIVE COMMON MARMOSETS (*CALLITHRIX JACCHUS*).

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Introduction

Common marmoset monkeys (*Callithrix jacchus*) are small South American primates. In the wild, marmosets average 320 to 336 grams (Araujo, et al., 2000). The average weight of captive animals ranges from 283 to 530 grams, with most animals historically being in the range of 350-400 grams. However, we have observed a consistent increase in the number of high-weight, high-fat animals in our colony over a 14 year period. Mean early adult weight in our colony is now close to exceeding 400g, and the proportion of adult animals with body weights above 450g has greatly increased. Although mean birth weight of colony infants has not significantly increased over the past 14 years, litter size has increased. Previously the modal litter size was twins; now it is triplets. This result is linked to the increase in mean maternal prepregnancy mass (Tardif and Jaquish, 1997). Adjusted for litter size, birth weight has increased, with mean triplet birth weight being almost equal to the mean twin birth weight of the past. Similar to the human experience, obesity is not just an adult condition in common marmosets. We have seen a steady increase in mean body weight among young animals in the colony, with many animals achieving what would be considered early adult weight while they are still juveniles.

In this study we investigated the development of adiposity in captive common marmosets from birth through 6 months of age in order to determine the age at which patterns of growth begin to diverge between lean and fat infant marmosets.

Methods

We report data from birth through 6 months on body weight and lean and fat mass for 18 marmosets, 8 considered to be lean and 10 considered to be fat (>15% body fat) at 6 months of age. Infants were weighed within 48 hours of birth (Day 1 wt), and then approximately every two weeks until 6 months of age; lean and fat mass were estimated by quantitative magnetic resonance at 1, 2 and 6 months. Body mass and age were transformed by the natural logarithm function, and the difference in ln(body mass) between the two groups was tested using ANCOVA, with ln(age) as the covariate. Differences between the two groups in body mass at specific time periods (day 1, months 1, 2, 3, and 6) were tested by ANOVA. Differences in lean and fat mass and the ratio of fat-to-lean at 1, 2 and 6 months were tested by ANOVA. The ratio of the changes in fat and lean mass between month 1 and month 6 was tested by ANOVA.

Results

Mean Day 1 wt was 31g and did not differ between groups. Similarly, the two groups were not significantly different in body mass at either 1, 2 or 3 months of age. However, at 6 months juveniles classified as fat weighed more (327g vs 215g, $F(1,16)=44.793$, $p<.001$) and had both greater lean mass (215g vs 171g, $F(1,14)=24.177$, $P<.001$) as well as fat mass (64g vs 19g, $F(1,14)=85.637$, $p<.001$). The relative gain in fat compared to lean mass over the first 6 months of life was greater in fat subjects (0.319 vs 0.078, $F(1,14)=117.7$, $p<.001$). The relationship between fat and lean mass changed between 1 and 6 months; in lean subjects it decreased while in fat subjects it increased (Table 1). By 2 months fat-to-lean mass ratio was significantly greater in fat subjects ($F(1,13)=6.72$, $p=.022$).

Interestingly, Day 1 wt was associated with the gain in fat mass from 1 to 6 months in fat subjects ($r=.857$, $p=.007$); but not in lean subjects ($r=.485$, $p=.223$).

Discussion

Excess adiposity in captive marmosets appears to develop as early as 2 months of age, and may be affected by birth condition, implying possible in utero effects.

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Table 1. Mean body weight, growth rate, lean and fat mass, and the ratio of fat-to-lean at different ages in common marmoset infants.

	1 month		2 months		6 months	
	Lean	Fat	Lean	Fat	Lean	Fat
Body weight	64.1	67.5	112.5	112.1	215.3	326.6
Lean mass	49.5	52.5	93.0	89.2	170.6	215
Fat mass	8.6	10.7	12.9	16.8	19.4	63.5
Fat:lean mass	.173	.203	.139	.187	.113	.294
Percent body fat	14.3%	16.1%	12.6%	14.5%	9.0%	20.10%

NUTRITIONAL CAUSES OF COMMON GIRAFFE HEALTH ISSUES AND HOW THEY'RE RELATED

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and Marcus Clauss. Dr. med. vet., M.Sc., Dipl. E.C.V.C.N.[2]

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According to a recent survey, the most common health problems encountered by captive giraffe are hoof overgrowth, chronic wasting and energy malnutrition, urolithiasis, digestive disorders, peracute mortality, dental disease, trauma, and arthritis. Additionally, giraffe in captivity have a tendency to demonstrate inverted serum calcium:phosphorus ratios[1]. Mineral absorption and excretion in ruminants are affected by dietary carbohydrate and forage sources.[2,3] Feeding practices in zoos vary widely and feeds offered to captive giraffe are significantly different from those encountered in the wild. This review will describe the digestive physiology of browsers in comparison to grazers, the common health problems exhibited by captive giraffe, and the potential for dietary imbalances to affect and cause the majority of these problems.

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NORTH CAROLINA ZOO GORILLA CHOW-FREE DIET: A KEEPER'S UPDATE

Aaron Jesue

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Since June 2008, the gorillas at the North Carolina Zoo have been offered a chow-free diet. The goal of this diet change was to try to improve problems like cardiovascular disease, obesity, and undesirable behaviors that are often seen in captive gorillas. Through 2008, and into 2009, the NC Zoo gorillas were fed this new diet and we observed a number of positive behavioral and physical changes in the group. Over the course of 2009, unrelated to the diet change, numerous health complications, medical procedures, and the eventual deaths of our three female gorillas have led the gorillas and keepers to go through numerous changes. In early 2010, the zoo received two new female gorillas. These females were transitioned to the chow-free diet. The zoo's gorilla troop now consists of 1.2 gorillas: Nkosi, 19 year old male silverback, Acacia, 15 year old female, and Jamani, 11 year old female. While the chow-free diet has remained essentially the same over the last two and a half years, we have also adjusted the diet based on preferences of the new animals, and fine tuned the consumption of the diet following a comprehensive nutritional review by a nutritionist. Other changes to the diet include incorporation of a weekly diet rotation and preparation for the possibility of further diet adjustments to accommodate a breeding recommendation for Nkosi and Jamani. While not without its challenges, the chow-free diet has been beneficial for the NC Zoo gorillas. The NC Zoo gorilla keepers continue to monitor the gorillas' progress on the chow-free diet and make additional changes to troop management and diet composition as needed.

PRELIMINARY BEHAVIOR AND HEALTH EFFECTS OF A BISCUIT-FREE DIET IN CAPTIVE GORILLAS

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All animals have adaptations for manipulating and digesting the food available in their natural habitats. In captive environments, however, it can be difficult to provide diets that are both nutritionally and functionally analogous to natural diets. In the wild, western lowland gorillas (*Gorilla gorilla gorilla*) travel long distances while foraging and consume a diet based primarily on large quantities of green plant material. This natural diet is high in fiber and low in caloric density. In contrast, many gorillas in captivity consume a diet that has low volume, low fiber and is calorically dense. The captive diets commonly contain significant amounts of vitamin and mineral fortified biscuits and gels, vegetables, domesticated fruit and browse. Some items (biscuits, potatoes and fruits) commonly used in captive gorilla diets contain high levels of starch and sugars, which are predominantly low in the natural diet of gorillas. Previous research suggests that species with significant gastro-intestinal adaptations for microbial fermentation (such as gorillas) can produce both a localized and systemic inflammatory response to the consumption of large amounts of dietary starch. Such inflammation has been linked to the development of heart disease in humans, which is also a major health problem in the captive gorilla population.

It is hypothesized that the relatively high caloric density of these diets contributes to the observed low levels of feeding and foraging activity in captive versus wild gorillas. Typical captive gorilla diets, concurrent with low levels of activity, may also lead to obesity, undesirable behaviors and other health problems.

In response to these issues, we implemented a biscuit-free diet (low or free of biscuit, gels and fruit) at five institutions: North Carolina Zoological Garden, Cleveland Metroparks Zoo, Woodland Park Zoo, Toronto Zoo and Columbus Zoo and Aquarium. We hypothesized that this diet change would reduce abnormal behaviors such as regurgitation and reingestion, decrease time spent inactive and increase time spent feeding/foraging/digesting. We also predicted that this diet change would reduce risk factors similarly associated with heart disease in humans such as biomarkers of obesity, insulin fluctuations,

increased cholesterol, and inflammation. Preliminary results of the biscuit-free diet at North Carolina Zoological Garden and Cleveland Metroparks Zoo revealed an elimination of regurgitation and reingestion behavior (R/R) and a significant reduction of hair-plucking behavior. A significant reduction in R/R was observed at Toronto Zoo and for some individuals at Columbus Zoo and Aquarium and Woodland Park Zoo. We did, however, observe an increase in coprophagy for many individuals following the diet change. A 10% increase in time spent feeding/foraging was observed at Cleveland Metroparks Zoo and North Carolina Zoological Garden but this increase was smaller in magnitude at the other three institutions. Overall time spent inactive was not consistently decreased with this diet change, which could reflect the need for a longer digestion period. Preliminary health analyses from serum collected before and after the diet change at North Carolina Zoological Garden indicate that biomarkers of obesity, insulin, cholesterol, and some inflammatory markers were reduced after the diet change. Future research will examine these biomarkers in a greater number of individuals from our participating institutions to determine if the results remain consistent with these preliminary findings.

SIXTH CRISSEY ZOOLOGICAL NUTRITION SYMPOSIUM

CONUNDRUM II

Do Plastics Used in Feeding and Storage Pose a Reproductive Risk?

Michael K. Stoskopf

The potential health effects of plasticizers have received considerable attention in popular press in recent years. Bisphenol A in particular has been presented as having a variety of health impacts with reports ranging from feminization of fish to association with miscarriages in humans. The National Toxicology Program of the NIEHS reached the following conclusions on the possible effects of current exposures to bisphenol A on human development and reproduction: *some* concern for effects on the brain, behavior, and prostate gland in fetuses, infants, and children; *minimal* concern for effects on the mammary gland and an earlier age for puberty for females; *negligible* concern that exposure of pregnant women will result in fetal or neonatal mortality, birth defects, or reduced birth weight and growth in offspring or reproductive effects in non-occupationally exposed adults. How concerned should we be about exposure of our animals to BPA or other chemicals in plastics?

KEY NOTE SPEAKER

SIXTH CRISSEY ZOOLOGICAL NUTRITION SYMPOSIUM

OLAV OFTEDAL, Ph.D.
Emeritus Scientist
Smithsonian Research Center

Dr. Olav Oftedal is an internationally respected comparative nutritionist interested in the study of nutritional ecology, food composition, digestive function, nutrient metabolism and nutrient export into eggs and milk for the young.

Over his career he has had a particular interest in interactions of nutrition and reproduction, including the evolution of lactation strategies in mammals. He has worked on milk composition and yield of a wide variety of mammals, including seals, bears, ungulates, primates, bats and rodents.

THE ORIGIN OF LACTATION AND THE EVOLUTION OF LACTATION STRATEGIES

Olav T. Oftedal PhD

Smithsonian Environmental Research Center, Edgewater MD

Lactation is a universal characteristic of mammalian reproduction that allows the young to reach a large size before they need to feed for themselves. This has been important to many aspects of mammalian life histories, and underlies the ability of mammals to specialize on adult foods that are otherwise unsuitable for rapidly growing offspring. Lactation appears to have an ancient origin within the synapsid lineages that led, in the Mesozoic, to the mammalian radiations. Mammary secretion was probably first important as a supplement to maintain water and nutrient balances of parchment-shelled eggs, and included antimicrobial compounds to protect eggs against microbial attack. Based on modes of secretion and ontogenetic evidence, mammary glands appear to have derived from apocrine-like skin glands. Many of the secretory pathways involved in milk secretion appear to have evolved from antimicrobial functions. The provision of nutrients via mammary secretions allowed a reduction in egg nutrient mass, which in turn was central to the miniaturization that occurred among Triassic mammaliaforms. Among extant mammals, monotremes lay and incubate small eggs, and milk secretion develops prior to egg-laying in tubular glands in a mammary patch or 'areola' that is located in the echidna pouch or platypus incubatorium. Marsupials also produce very small neonates, but the young are liveborn and attach to a nipple. In both monotremes and marsupials, lactation is of long duration, and there are tremendous changes in mammary size and milk composition, keeping pace with the changing nutritional and physiologic needs of the developing young. In eutherian mammals the evolution of placental nutrient transfers has eliminated the earlier stages of lactation, but this appears to have allowed greater customization of milk composition to the constraints faced by mothers and to the diverse needs of offspring. Examples of the remarkable lactation strategies of some terrestrial and marine mammals will be discussed in relation to their life histories. Further research is needed on the relationship of milk composition to phylogeny, neonatal physiology, nutritional needs, and the metabolic constraints faced by lactating females in very different natural environments.

GEOMETRY AND THE NATURE OF NUTRITION

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Nutrition is a complex process of matching multiple and dynamic nutrient needs to variable, changing and sometimes hostile foods. And yet animals have evolved highly effective regulatory strategies for dealing with this complexity. For biologists, however, the challenge remains of how to understand – and, in many cases (e.g. human nutrition, pet nutrition, zoo nutrition, conservation biology) to manage - these complex processes. In this talk I will show how simple geometry can be used for this. I will introduce the basic concepts of a geometric framework for nutrition, and present examples demonstrating how it has been applied to a number of biological problems, with an emphasis on the relationship between longevity and reproduction. To underscore the generality of the geometric framework for nutrition, I will present examples spanning insects to humans.

DIETARY PRACTICES FOR HAND-REARING OF INFANT CHIMPANZEE (*Pan troglodytes)* AT THE NORTH CAROLINA ZOO

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Jennifer Ireland, B.A. Animal Management Supervisor
North Carolina Zoological Park, 4401 Zoo Parkway, Asheboro, North Carolina 27203

On August 2, 2010 a primiparous chimpanzee gave birth to a female infant. Maternal competence was questionable as improper positioning of the infant prevented the infant from nursing. On days 0 and 1 of the infant's life, the mother was anesthetized and the infant was allowed to nurse. On days 2 through 4 keepers were able to periodically bottle feed the infant through the caging. On day 5 the decision was made to pull the infant for hand rearing as food presentation to the infant became unreliable and required considerable cooperation from the mother.

The infant chimpanzee was put on a scheduled feeding regime consisting of human infant formula. Consumption, fecal output and daily weights were recorded. Adjustments to the amount and type of formula offered were made based on consumption, fecal output, weight, behavior, and social milestones.

Traditionally chimpanzee infants are introduced back to adults between six to eighteen months of age. With the permission of the chimpanzee SSP plans were made for reintroduction at approximately 4 months dependent on the physical development and mobility of the infant. Because of this, traditional methods for food introduction and feeding schedules were accelerated so that the infant was more independent and ready to consume novel foods at an earlier age.

PROXIMATE COMPOSITION OF THE MILK OF THE OWL MONKEY.

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Lactation is a defining characteristic of mammals that is unfortunately understudied. All mammals lactate, however there are numerous lactation strategies that have evolved. The lactation strategy of a species includes the duration of lactation, the frequency of suckling by the infant, the volume of milk produced daily by the mother, and milk composition. Primates in general have long lactations, nurse frequently, and produce large amounts of dilute milk.

Owl monkeys, members of the genus *Aotus*, are New World monkeys that inhabit the forests of Central and South America. Owl monkeys inhabit a wide range of forests, from tropical to dry, and reside in a variety of elevations. They are the only nocturnal monkey. Owl monkeys are not sexually dimorphic and weigh slightly over a kilogram. In this study we examined the proximate milk composition from captive owl monkeys and compared the results to those from other New World monkey species that have been studied previously.

Methods

Milk samples were from 28 lactating mothers, one sample from each female, and were collected from between one and four months post partum. Proximate analyses of the milk samples were performed at the Nutrition Laboratory at the Smithsonian Institution National Zoological Park. Dry matter was measured by oven dying samples at 100°C for three hours. Total lipid was measured by sequential extractions with ethanol, diethyl ether, and petroleum ether in a micro modification of the Rose-Gottleib procedure. Sugar was assayed through the phenol- sulfuric acid method, using lactose monohydrate as the standard (Dubois et al., 1956; Marier & Boulet, 1959), with results expressed on an anhydrous lactose basis. Nitrogen is assayed by a CHN elemental gas analyzer (Model 2400, Perkin-Elmer, Norwalk, CT) that provides a rapid and accurate method of assaying total nitrogen (TN) in each sample. Crude protein (CP) is estimated from the TN as 6.38 x total nitrogen. Gross energy (GE) of milk was calculated using 9.11 kcal/g for fat, 3.95 kcal/g for sugar, and 5.86 kcal/g for CP. The mg of fat, sugar and CP in a kcal of milk was calculated for each sample. Results are presented as mean ± SEM.

Results and Discussion

Infant age ranged from 30 to 118 days old; thus all samples were past colostrum production and not near the end of lactation (weaning does not occur until after 5 months of age; Dixon and Fleming, 1981.) On average, samples contained 83.33% water. The average percentages for each component are displayed below in Table 1. On a per gram basis, fat was the most variable constituent, while sugar and protein were relatively constant (Table 1). There were no significant correlations in milk constituents with infant age. Milk GE did not vary systematically with infant age, but did vary substantially among females (Figure 1). On an energy basis (mg/kcal), both sugar and fat were more variable than was CP (Table 1). As expected, the mg/kcal of fat increased with increasing milk GE ($r=.919$, $p=.001$) while the mg/kcal of sugar decreased ($r=-.967$, $p=.001$). This pattern is consistent with results from other New

World monkey species, including common marmosets, squirrel monkeys and capuchins (Power et al., 2002; 2008; Milligan et al., 2008; Milligan, 2010). In these species the mg/kcal of CP did not vary with milk GE; however, in owl monkeys the mg/kcal of CP significantly declined with increasing GE ($r=-.690$, $p=.001$).

The values for fat, sugar, CP and GE for captive owl monkey milk are similar to those found for captive common marmosets (Power et al., 2002) and squirrel monkeys (Milligan et al., 2008), and virtually identical to the results for captive capuchin monkeys (Milligan, 2010). The mg of CP per kcal was significantly lower, on average, than values for marmosets and squirrel monkeys, and not different from value from capuchins. Protein content of milk expressed on a per-energy basis has been suggested to be associated with growth rate in primates (Power et al., 2008) suggesting that owl monkeys (and capuchins) might have a slower growth rate than do common marmosets or squirrel monkeys.

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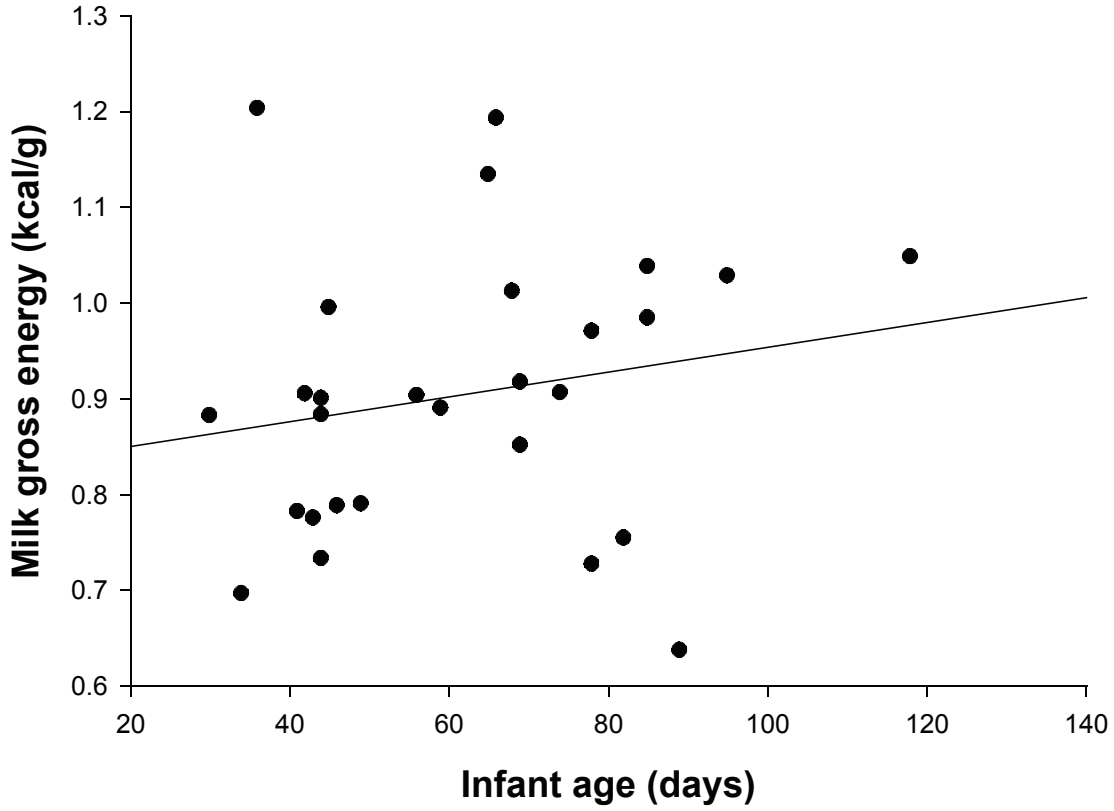
Table 1: Mean values of dry matter (DM), fat, sugar, crude protein (CP), and gross energy (GE) in *Aotus* milk.

Components	Sample Number (N)	Mean	Range
DM (%)	28	16.48 ± 0.33	13.44 – 20.25
Fat (%)	28	5.10 ± 0.29	2.28 – 7.99
Sugar (%)	28	7.03 ± 0.07	5.69 – 7.52
CP (%)	28	2.77 ± 0.06	2.25 – 3.69
Gross Energy (Kcal/g)	28	0.90 ± 0.03	0.637 – 1.203
mg CP/ Kcal	28	31.12 ± 0.92	23.19 – 47.30
mg fat/ Kcal	28	55.11 ± 1.67	32.78 – 67.55
mg sugar/ Kcal	28	79.90 ± 2.79	54.57 – 113.50

Figure 1. Milk gross energy (GE) versus infant age. Regression is not significant (p=.324). Mean milk GE = 0.90 kcal/g. Each point is from a different female.



HAND-REARING NEONATAL EASTERN COTTONTAILS - A SURVEY OF METHODS USED FOR SUCCESS

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Most young Eastern Cottontails admitted for rehabilitation as orphans have either been kidnapped (typically well-meaning members of the public are not knowledgeable on the natural history of rabbits and are not aware that doe cottontails do not incubate nests but only visit to nurse young every 12 to 24 hours) have fallen prey to domestic pets, or have had their nest disturbed/destroyed by lawnmowers, kids or pets. As a prey, flight or fight species, Eastern Cottontails are highly susceptible to stress and tend to not adapt well to captive care. Suckling rabbits are unique in that typical gut flora is not present in their relatively high pH gastric environment, and are therefore vulnerable to bacterial enteritis. Challenges such as these are why there is not more documented and/or anecdotal success in the hand-rearing of neonatal Eastern Cottontails.

The Valerie H Schindler Wildlife Rehabilitation Center at the North Carolina Zoo admits, on average, 125 Eastern Cottontails every year, of those approximately 30% are neonates less than 3 days old. Historically the center's success of hand-rearing neonatal Eastern Cottontails to release has been less than 1%. In March 2010 a survey was sent out to wildlife rehabilitators and wildlife rehabilitation facilities through both direct emailing as well as posting on relevant list serves; the questionnaire addressed nutrition, nursing techniques, as well as husbandry and caregiver information for the hand-rearing of neonatal Eastern Cottontail. The information gathered through the results of this survey led to protocol changes in hand-rearing techniques as well as formula supplementation increasing the center's rearing to release to 15% success.

SIXTH CRISSEY ZOOLOGICAL NUTRITION SYMPOSIUM

CONUNDRUM III

Can Contraception Affect Nutritional Husbandry?

Alejandra Renjifo-McComb

Reproductive management in captive animal populations is necessary to maintain genetic diversity, prevent inbreeding and sustain appropriate collection size.

In the case of many exotic species, group exhibits housing multiple individuals of varying reproductive statuses necessitates the use of contraception methods.

There are several manners in which the rate of conception can be controlled. Some examples of contraceptive strategies include hormone dosing in the feed, hormonal implants or injections, anti-reproduction vaccines, and reversible & non-reversible sterilization (penile diversion, vasectomy, tubal ligation, castration, ovariohysterectomy).

Are there instances where side effects from the use of contraception methods require the nutritionist to adjust the nutritional management for the welfare of the animal?

COMPARISON OF IONIZED CALCIUM, PARATHYROID HORMONE, AND 25-HYDROXYVITAMIN D IN REHABILITATING AND HEALTHY WILD GREEN SEA TURTLES (*CHELONIA MYDAS*)

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Rehabilitating green sea turtles (*Chelonia mydas*, n = 10) were diagnosed with markedly inverted calcium:phosphorous (Ca:P) ratios on pre-release plasma biochemistries. A clinical diagnostic investigation was conducted to assess ionized calcium (iCa), 25-hydroxyvitamin D (Vit D), and parathyroid hormone (PTH) levels in the affected animals. Because normal values for iCa, Vit D, and PTH have not been reported for wild green sea turtles in this region, healthy wild turtles (n = 10) that were incidentally captured in commercial fishing nets along the North Carolina coast were also evaluated. Median levels of iCa, PTH, and Vit D for rehabilitating turtles were 0.63 mmol/L, 2.95 pmol/L, and 27.5 nmol/L, respectively, and for wild turtles were 1.05 mmol/L, 0.75 pmol/L, and 36 nmol/L, respectively. Significant differences were found between the two populations for Ca, P, Ca:P, iCa, and PTH. Vit D values did not differ between the two populations, suggesting that rehabilitating turtles maintain adequate levels. However, Ca and iCa values were significantly lower and PTH and P were significantly higher in rehabilitating turtles than in wild turtles. This suggests that dietary requirements are not being met in the captive population, with UV exposure insufficient to compensate. The values presented here for iCa, Vit D, and PTH from the healthy wild population may serve as references for future rehabilitating green sea turtles.

CATABOLIC PROCESSES OF A FRESHWATER MUSSEL, *ELLIPTIO COMPLANATA*

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Despite a number of investigations to determine what comprises the diet of freshwater mussels, many questions still remain about what mussels eat. However, inadequate nutrition due to resource competition and habitat change are considered factors in the failure of freshwater mussel populations to thrive (Bogan 1993). Also, many toxicologic studies using freshwater mussels fast study animals for up to three weeks at a time, resulting in a potentially catabolic state which may alter physiologic responses to toxicants. By defining catabolic processes in freshwater mussels, we can highlight metabolic markers associated with starvation, suggest alternate pathways mussels use to meet demand in resource limited situations and determine whether fasting is appropriate in determining mussel response to a pollutant. Through use of ^{1}H-nuclear magnetic resonance (NMR) spectroscopy, the metabolic profile of fed and fasted *Elliptio complanata* will be compared. Preliminary investigations show that *E. complanata* produces the polyamine putrescine in varying quantities dependent upon tissue examined and metabolic state. The possibility that the presence of putrescine in freshwater mussels may suggest an alternate use of the ornithine-urea cycle where ornithine decarboxylase is used to convert ornithine to putrescine in these ammonotelic species will be discussed.

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THE EFFECT OF DIET ON FERMENTATION AND MICROBIAL STRATEGIES IN PRIMATE AND CARNIVORE SPECIES

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Nutrition as a form of preventative medicine and a foundation for optimum health has great importance in captive animal settings. Primates fed diets high in starch and insoluble carbohydrates have an increased incidence of obesity and diabetes compared to those consuming diets higher in fiber. The dietary discrepancy between native and captive diet offerings has also had quantitative repercussions in microbial activity and diversity in primate species (Kisidayova et al., 2009; Nakamura et al., 2009). Microbial populations play a key role in the process of digestion for nutrient use by the animal (Stevens and Hume, 1998). For example, animals that exhibit folivorous tendencies (consume diets higher in structural carbohydrates) would be expected to exhibit different fecal microbial profiles from animals that have more frugivorous (non-structural carbohydrate-based) feeding strategies.

This project aims to compare the community composition and fermentation activity of gastrointestinal bacteria isolated from Western lowland gorilla (*Gorilla gorilla gorilla)*, chimpanzee (*Pan troglodytes)*, Hamadryas baboon (*Papio hamadryas)*, and binturong (*Arctictis binturong)* fecal samples. The primate species were chosen to represent feeding strategies across the omnivorous spectrum. In the wild, Western lowland gorillas consume a diet comprised mainly of leaves and plant material, while free-ranging chimpanzees forage for fruits; both supplement their diet with high-protein insects and small prey. Hamadryas baboons are more opportunistic, feeding on a wider variety of fruits and vegetation and a higher percentage of small prey. Binturongs are carnivores, but like chimpanzees they consume up to 90% fruit *in situ.* Host behavior and gastrointestinal morphology are suspected to have an impact on the microbiota associated with each species. By characterizing the demographics of bacterial populations as well as their fermentation profiles, we hope to better understand the ramifications of diet on host animal health. These relationships may have further implications for management in captivity.

All primate species were housed at the North Carolina Zoo, Asheboro, NC. Binturongs were kept at the Carolina Tiger Rescue, Pittsboro, NC. All animals were fed a predetermined diet for two weeks prior to sample collection. Fecal specimens were collected within two hours of defecation and sealed in labeled Ziplock® bags labeled with animal species, donor ID, and the date. Gorillas and binturongs were housed individually, so their samples were labeled by name. Baboons and chimpanzees were housed in troops, so each fresh sample was numbered for tracking purposes although the individual donor was unknown. Filled sample bags were placed into pre-warmed vacuum Thermos® flasks and immediately transported to the lab. Upon arrival, fresh feces were quantitatively mixed with media and buffer in a blender; buffer composition matched the formula developed by Sunvold et al (1995). Fecal inoculums were added to culture bottles containing pre-weighed dietary substrate, purged with CO_2 and sealed with crimp tops. Culture bottles were placed in 37°C water baths for 24h. At the end of 24h, all bottles were removed from the water bath and placed on ice to stop microbial activity. Methane concentration in the bottle headspace was determined using gas chromatography immediately prior to removing the caps. A 5-mL culture sample was processed for analysis of volatile fatty acids (VFAs) using capillary gas-liquid chromatography. The remaining residue was processed to determine neutral detergent fiber (NDF) and acid detergent fiber (ADF) sequentially using an Ankom fiber extractor (Ankom Technologies, Fairport, NY) according to the method of Van Soest et al. (1991) without amylase and sodium sulfite. Fermentation data were

analyzed using the Proc Mixed procedure of SAS®, Cary, NC.

An aliquot of fresh fecal specimens was stored at -80°C for subsequent determination of microbial profiles. Microbial DNA was extracted using the QIAamp Stool Mini Kit, pooled across individuals, and a region of the *cpn60* gene was amplified using PCR. The *cpn60* gene was chosen for bacterial identification because it is more highly conserved than the 16S ribosomal RNA region that is conventionally used (Hill et al., 2002). The *cpn60* amplicons were cloned into the PGem Easy vector and transformed into competent *E. coli* cells to produce a library for each represented species. Cells were plated and 384 white clones were isolated and processed for gene sequencing. Sequences were trimmed to eliminate vector overhangs and the *cpn60* sequences were compared to the BLAST database for microbial species identification.

Methane concentration was higher ($P < 0.0006$) for gorillas and chimpanzees than for baboons. Gorillas also exhibited higher ($P < 0.0001$) acetate to propionate (A:P) ratios than chimpanzees and baboons. This difference in A:P production may result from the gorilla diet being higher in structural carbohydrates than the chimpanzee and baboon diets, which both contain about 75% pelleted feed. In a previous study binturongs exhibited no fermentation activity. Microbiota identified in chimpanzee feces represented predominantly uncultured species, most of which have been previously isolated from pig feces. The majority of identified microbes in binturong samples belonged to the Family *Leuconostocaceae*. These markedly distinct microbial populations may corroborate the lack of fermentation products in binturongs. Construction of the gorilla and baboon *cpn60* libraries is underway. Our findings help explain dietary impact on fermentation patterns observed across different animal species and corresponding changes in their respective microflora. Gastrointestinal bacteria play a key role in the digestive process and contribute to overall host health. Further research is needed to compare the microbiomes of captive individuals with the internal dynamics of their wild counterparts, in order to more fully replicate their existence *in situ*.

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SIXTH CRISSEY ZOOLOGICAL NUTRITION SYMPOSIUM

CONUNDRUM IV

Should Captive Primate Diets have Their Starch and Sugar Levels Reduced?

Kimberly Ange-van Heugten

In the last several years, various starch free and altered sugar content feeds have been produced for captive primates. There is a division within the scientific community as to whether this is simply a new diet fad or if the starch and sugars supplied in many commercial diets are truly unhealthy. Historically, many researchers have indicated that very little starch was present in the natural foods consumed, particularly by Great Apes, and that the sugar profile is quite different within natural diet items versus cultivated ones.

New researchers have postulated that previous free-ranging diet studies may have underestimated the starch and sugars primates consume (i.e.: plant samples should be fully examined through all seasons and stages of growth to give true estimates of nutrients consumed).
Thus, should starch be removed from captive diets and should the sugar profiles be changed OR are these diet changes as likely to cause new problem as they are to prevent old ones?

Gastrointestinal transit time and its relationship to fasting protocols utilized prior to anesthetic immobilizations in the captive chimpanzee, *Pan troglodytes*

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Anesthetic immobilization in captive primates often involves separating individuals from their social group for overnight fasting to minimize the risks associated with anesthesia, including regurgitation and aspiration. Both lengthy social separation and prolonged fasting time contribute to heightened agitation amongst the animals, making immobilizations more challenging. To address that concern, the current practice of abbreviated pre-anesthetic fasting times in humans was reviewed and guided the objectives of this study, which included: 1) identifying the gastric emptying time (GET) and total gastrointestinal transit time (GTT) of chimpanzees, *Pan troglodytes*; 2) determining the shortest safe fasting time for anesthetic administration. Eleven (6 female, 5 male) adult (9-41 year old) chimpanzees at the North Carolina Zoo were utilized in a pilot study to measure GET and GTT. Seven of the 11 chimps immobilized for annual physical exams were fed barium impregnated polyethylene spheres (BIPS) to measure GET. To determine GTT, all 11 chimps were individually fed a color dye marker and fecal passage was observed. Results of the two study designs revealed that the GET approximated a time greater than 3 hours but less than 16 hours and that the mean GTT was 16.5 hours. These findings suggest that a fast closer to 3 hours would allow for complete gastric emptying and could replace the current overnight fast of near 16 hours, helping to minimize complications associated with lengthy pre-anesthetic fasting protocols in captive chimpanzees.

DIETS OF CAPTIVE SAND TIGER SHARKS (*CARCHARIAS TAURUS*)

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Sand Tiger Sharks (*Carcharias taurus)* are a commonly exhibited elasmobranch species. Relatively little is known about the feeding habits of these sharks in the wild or their nutritional requirements. Several syndromes are described in captive sand tiger sharks that have the potential to be due at least in part to nutritional factors. Our objective is to describe the feeding protocols used by aquaria that hold sand tiger sharks on display to serve as a foundation for further investigations into sand tiger shark nutritional requirements. Curators of nine aquariums with long term displays of sand tiger sharks were interviewed and asked pre-determined questions pertaining to the feeding protocols for their sand tiger sharks. The information collected on diet composition, frequency of feeding, food preparation and diet delivery methods were examined for trends and in relationship to the gender and time in captivity.

DIET INFLUENCE ON URINE PARAMETERS AND UROLITHIASIS IN CAPTIVE GIRAFFE

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Urolithiasis occurs in many ruminants and is diet related. Urine parameters, including pH, specific gravity, and phosphorous may promote uroliths. Various pH levels influence uroliths: silica stones in acidic, ammonium urate stones at neutral, calcium oxalate stones at variable pH, and struvite stones at alkaline, especially with a specific gravity ≥ 1.025. High concentrate diets are linked to struvite (magnesium ammonium phosphate) uroliths. Water buffalo (*Bubalus bubalis*) in China fed cottonseed meal produced struvite crystals (Wang et al 1997). High concentrates fed at 2.5% of animal body weight showed urolith formation within two months in cattle (Radostits et al 2000). Obstructive urolithiasis can cause death, especially in males due to anatomy. Diet management and adequate water intake may prevent or deter uroliths. Surgery can be done to remove calculi from the bladder and urethra and in some cases lithotripsy may be of a benefit.

A 12 year-old, male giraffe (*Giraffa camelopardalis*) had stranguria, polkakiuria, and hematuria at the Oakland Zoo in 2010. Magnesium ammonium phosphate crystals and blood were present and urine phosphorous was 30.9 mg/dl. Also, 21 year-old female was asymptomatic with magnesium ammonium phosphate crystals and urine phosphorous value of 11.8 mg/dl. Diet changes were made and the phosphorous levels dropped. Subsequent urine samples were negative for crystals and blood. The pH and specific gravity did not change. Diet change is attributed to ending the symptoms.

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

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Supplying an adequate energy supply is imperative to optimizing the husbandry of captive populations of North American river otters (*Lontra canadensis*). While it is known that the metabolic rate of mustelids is higher than that of other mammals (Iversen 197[2)], calculating a dietary ration from the intake of gross energy of the diet is insufficient. Knowledge surrounding the efficiency of the digestibility of different captive diets items is essential because the ingested gross energy cannot be entirely utilized. Diets currently provided to captive North American river otter are not universal with some providing a more natural free-ranging diet (fish, shellfish, rodents, fruit, vegetables, chicken) and others opting for a prepared diet (Science diet® Feline diet, Topeka, KS), Nebraska Brand® Feline diet North Platte, NE, Iams® Feline diet, Dayton, OH, Mazuri® Polar Bear diet, Saint Paul, MN). Energy content, proximate nutrient values and apparent digestibility associated with a more fish based diet has not been quantified for the North American river otter, but is important if we are to provide a diet which is sufficient for maintenance, growth and reproduction in this highly active species. The primary aim of this study is to quantify the exact diets being fed to the nine North American river otters held at the three North Carolina institutions (North Carolina Zoo, Asheboro, NC, North Carolina Aquarium at Pine Knoll Shores, Pine Knoll Shores, NC, North Carolina Aquarium on Roanoke Island, Manteo, NC) and examine their proximate nutrient values (crude protein, crude fat, crude fiber, moisture, ash, calcium, phosphorus, magnesium, vitamin A, vitamin E and vitamin B_1). Secondarily we will investigate the digestible energy intake and determine the apparent digestibility of energy for these diets.

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CONCENTRATED FEED SELECTION FOR A MIXED SPECIES UNGULATE COLLECTION

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In the African portion of the North Carolina Zoo is a 40 acre exhibit home to multiple hoofstock species with varying feed strategies including browsers, grazers, and intermediate ruminants. In 2006 the collection was transitioned from ADF-16 feed to the Wild Herbivore products in an effort to improve the animals' health by addressing concerns of acidosis and "lumpy jaw". In the three years post transition, cases of nutritional issues subsided but the animal collection displayed poor body condition and pelts, most notably in the small grazers and intermediate species.

Several remedies were tested in this time period as an effort to improve the health and quality of life for our herds. Synthetic termite mounds were constructed to reduce the antelopes' grain being consumed by the newly introduced White rhinos. Although they successfully eliminated the competition for grain rations, weight issues persisted. Multiple feed delivery areas and an increase of grain offered had no effect on animal weights. Several supplements in various combinations of product were incorporated into the hoofstock diets without noticeable improvement and there was continued concern about meeting caloric needs through the winter weather.

A discussion in 2009 with the Mazuri representatives prompted a switch in products, moving away from the Wild Herbivore diets to a product known as Browser Breeder. Within months keepers noted not only an increase in consumption but body condition began to improve and winter weights were maintained. The beneficial effects were further magnified with better delivery methods for alfalfa and native browse plants to the exhibit.. By the summer of 2010 keepers noted all herds to be thriving with healthy weights and successful reproduction. While we continue to monitor the body condition of all animals on the Watani Plains, we acknowledge the need to understand and better address nutritional concerns for a mixed species exhibit that will meet the needs of a wide range of specialized herbivores.

A SLIPPERY SLOPE: BALANCING NUTRITION AND HUSBANDRY IN A PEREGRINE FALCON

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Proper nutrition is vital to normal growth, health, reproduction, and longevity in captive raptors. Presented is a case that highlights the importance of considering adequate nutrition along with husbandry matters to address health concerns in captive raptors. An adult female peregrine falcon was acquired from a breeding facility, along with several other birds, by a public aquarium for an interactive bird program. During an examination approximately 1 month after acquisition, the bird weighed 24% less than its acquisition weight and was in poor body condition. An evaluation of the diet found it consisted solely of 1-3-day-old chicks dusted with a vitamin supplement. Because of the inadequate nutritional value of chicks (eg. insufficient calcium), adjustments in diet included increased quantity of prey items fed and a shift from 1-3 day-old-chickens/quail to adult quail. The diet changes led to improvements in both the peregrine's body condition and its overall attitude. However, increased body condition combined with delayed improvements to perching conditions, led to worsening pododermatitis of both feet. This cases emphasizes the need to address both husbandry and nutritional factors in tandem in captive raptors.

FOOD SUPPLEMENT PIGMENT INTERFERENCE WITH COLORIMETRIC SEROLOGICAL HEALTH TESTS

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Carotenoids food supplements are often supplied in diets of zoo animals to enhance plumage coloring or to increase dietary provitamin A content. The carotenoids most often used in the supplements added to zoo animal feeds are Canthaxanthin, Astaxanthin, and Beta-carotene. Other naturally occurring carotenoids such as Zeaxanthin and Cryptoxanthin can be used. Carotenoids are tetraterpenoids with hydrocarbon chains of 40 carbon atoms, sometimes terminated with ring structures. Dry carotenoid powders or dissolved carotenoids range in color from pale-yellow to bright-red but absorb light in the blue range. They are produced by plants and in general vertebrates cannot synchronize carotenoids but have to obtain them in their food. Ingested carotenoids accumulate in various organs and structures, but can also be found in serum. The light absorbance properties of carotenoids in serum can interfere with colorimetric diagnostic tests, especially when the tests use optical readings taken on the wavelengths within the maximum absorption range of the carotenoids. The maximum light absorption of the five frequently used carotenoids in color enhancing food supplements ranges from 449 to 480 nanometers. This absorbance range can with various serum ELISA antibody tests, toxin assays, and enzyme assays. We will present a case where pigment supplementation in the feed resulted in serum accumulations of carotenoids sufficient to give false positive results on ELISA tests being used to screen flamingos for antibodies to avian influenza virus.

RUMINAL DEGRADATION OF PROTEIN & CARBOHYDRATE IN DOMESTIC & WILD RUMINANTS: OUT OF THE FOREST AND ONTO THE FARM.

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Wild ruminants make up 6 separate families in the animal kingdom, encompassing 193 species and an estimated 75.3 million animals worldwide (Hackmann, 2005). The ruminants' ability to subsist on grass via bacterial pre-gastric fermentation is the latest step on the digestive evolutionary ladder. Ruminant animals can be classified by digestive strategy and morphology as browsers, grazers, or an intermediate type feeder. As is implied by the name, grazing ruminants graze in areas with an abundance of grass species, have wider mouths, larger rumens per body size and slower passage rates; whereas browsers subsist mainly on forages, stems, and fruits, ingest smaller feed particles, have faster passage rates, greater salivary production and lack ruminal stratification. Intermediate feeders are able to switch between feeding strategies based on availability of feedstuff. Grazers and intermediate feeders are only found in two of the 6 families (*Bovidae*, *Cervidae*) but make up a majority of the wild ruminant population on the planet; however, 81 of the 193 species are browsers (Van Wieren, 1996). Therefore, in a diverse environment, such as in zoos, it stands to reason that many, if not a majority of species are likely to be browsing ruminants. Further, growing commercial elk and deer production in the U.S. and abroad means browser nutrition is becoming of major economic importance.

Historically, captive wild ruminants were fed similar to their domesticated cousins: cattle, sheep and goats; but none of these animals are strict browsers. This becomes a difficulty in captive settings due to the differences in digestive morphology. The excess dietary starch and lack of dietary fiber, characteristic of diets fed to domestic production ruminants, have been implicated in mortalities of several browsing ruminants. Resulting acidotic conditions would decrease food consumption and decrease nutrient absorption (Nagaraja and Titgemeyer, 2007). This is a plausible cause of acidosis and urolithiasis seen in deer (Woolf and Kradel, 1977; Woolf et al., 1976) and peracute mortality syndrome seen in captive giraffe, which demonstrate fat atrophy upon necropsy (Clauss et al., 2002; Potter and Clauss, 2009), due to energy loss and malnutrition. We would expect fiber-rich diets with lesser amounts of readily fermentable carbohydrates to be more reflective of diet selected by wild browsing ruminants (Dierenfeld et al., 2002; Kearney, 2005).

Recent research (Brooks, 2010; Brooks et al., 2010) using *in vitro* batch and continuous culture methods have shown microbial inoculum obtained from domestic and wild ruminants responds differently to differing levels of starch and carbohydrate. These studies utilized diet adapted mule deer (*Odocoileus hemionus*) and lactating dairy cows (*Bos taurus*) as comparison models. Both studies showed greater capacity for protein degradation in dairy cows compared to mule deer (Figure 1A & Table 1), while also showing greater capacity for fiber degradation in mule deer than in dairy cows as evidenced by greater acetate and butyrate production. Even with higher starch levels, pH measured in both studies remained above acidotic conditions (6.3 to 7.0). The batch culture study (Brooks et al., 2010) found as fermentation continued in the "closed" system, variations between diets became more pronounced, and total volatile fatty acids (Figure 1B) increased to beyond physiological levels by 48 h, indicating extended fermentation in this model began to lose physiological significance. In contrast, the

continuous culture system (Brooks, 2010) used a constant influx of nutrients, allowed for outflow of digesta, and was able to maintain conditions more similar to the live animal. This study found mule deer have higher acetate:propionate ratios (Table 1) and feeding a higher fiber/lower starch diet increased acetate:propionate ratios to values more similar to those measured in wild caught mule deer (Short, 1971) and other browsing African ruminants (Maloiy et al., 1982).

In conclusion, diets are continuing to be modified as more information becomes available on the nutritional content of the wild diet of browsing ruminants. The zoo industry, in collaboration with feed manufacturers and researchers, has made great strides in recent years to solve the nutritional problems plaguing browsing ruminants in captivity. Although much work is still left to be done, it is clear that accurate information on fermentation end-products of wild browsing ruminants requires the use of a browsing ruminant model. Perhaps with the rise in deer farming, this type of model will become more available. Until such time, researchers using domestic ruminant models for microbial digestion studies will need to be cautious when interpreting their results. They will also need to remember, you can take a Bongo out of the bush, but that still does not make him a cow.

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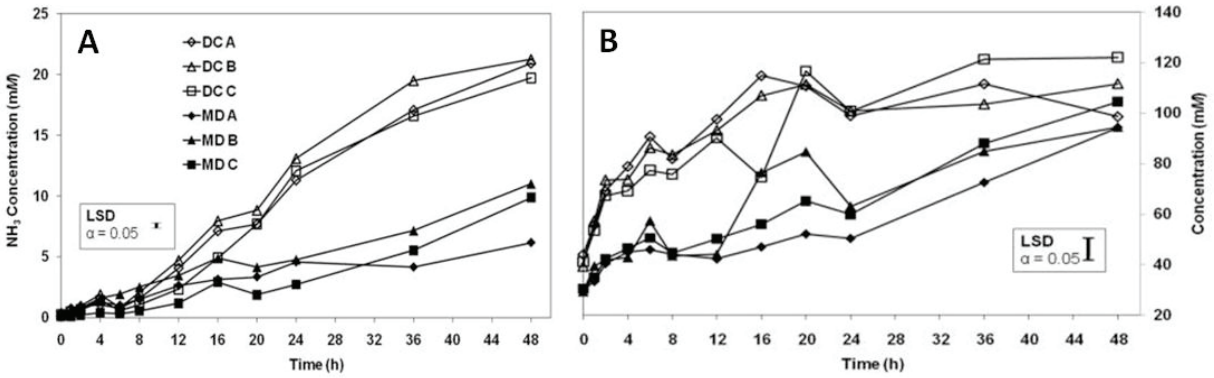

Figure 1. Ammonia (NH_3; A) and Total VFA (B) of three commercial exotic ruminant pelleted feeds of varying starch and fiber levels with alfalfa cubes (75/25 mix) using *in vitro* batch culture techniques with mule deer (*Odocoileus hemionus*; closed markers) or lactating dairy cow (*Bos taurus*; open markers) rumen fluid inoculum. Diet A = high starch/low NDF (forage fiber basis; ♦ and ◊); Diet B = high starch/low NDF (by-product fiber basis; ▲ and Δ); Diet C = Low starch/high NDF (■ and □).

Table 1. Microbial fermentation characteristics from digestion of three commercial exotic ruminant pelleted feeds of varying starch and fiber levels with alfalfa cubes[1] using a continuous culture system inoculated with mule deer (*Odocoileus hemionus*) or lactating dairy cow (*Bos taurus*) rumen fluid inoculum.

Sample 4 h post-feeding	Mule Deer Inoculum[2]			Dairy Cow Inoculum[2]				*P*-values[3]		
	Diet A	Diet B	Diet C	Diet A	Diet B	Diet C	SE	I	D	I x D
NH_3 (m*M*)	5.35	4.29	1.08	10.42	9.01	5.97	0.46	<0.01	<0.01	0.93
pH	6.59	6.57	6.55	6.66	6.56	6.58	0.03	0.21	0.08	0.42
A:P Ratio	4.81	4.52	5.49	3.58	3.39	4.00	0.24	<0.01	<0.01	0.75

[1] Dietary treatments consist of 75% pelleted feed and 25% alfalfa cubes.
[2] Diet A = High starch/low NDF (forage fiber basis); Diet B = High starch/low NDF (by-product fiber basis); Diet C = Low starch/high NDF
[3] I = Mule Deer inoculum vs. Dairy Cow inoculum; D = A vs. B vs. C; I x D = Interaction

EVALUATION OF THREE VITAMIN A SUPPLEMENTATION METHODS IN A CAPTIVE TOAD POPULATION

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Introduction

Successful conservation efforts for preserving anura populations have led to the creation of several captive colonies of threatened and endangered toads and frogs. Also, a significant number of non-endangered species are kept in private ownership as pets. Adequate diet formulation and provision of nutrients is necessary for optimum captive care as well, as to ensure thriving breeding programs of fit individuals for future release. Several medical instances of hypovitaminosis A have been reported for insectivorous anura receiving diets of predominantly commercially-raised invertebrate prey for a prolonged period of time. In particular, symptoms of eye lesions and anatomical changes to the buccal cavity have been observed. Chiricahua leopard frogs (*Rana chiricahuensis*) and bumblebee dart frogs (*Dendrobates leucomelas*) consuming diets lacking vitamin A developed swellings on the lower eyelids which were reduced in response to vitamin A treatments (Wright, 2005). In adult toads with low hepatic retinol, a reduced ability to capture live prey with the tongue, a condition termed "short-tongue syndrome" but more commonly known as lingual squamous metaplasia (LSM), was observed as occurring in conjunction with hypovitaminosis A in a captive population of Wyoming toads (*Bufo baxteri*) (Pessier et al., 2002). Two other toad species, Rocky Mountain boreal toads (*Bufo boreas boreas*) and Woodhouse's toads (*Bufo woodhousii*) have since been reported as showing symptoms of LSM (Pessier et al., 2005).

The diet of adult anura species is almost entirely carnivorous (Vitt and Caldwell, 2009). Evaluations of nutrients found in a variety of commercially-raised invertebrates, such as house crickets (*Acheta domesticus*), found them to be low in vitamin A (Dierenfeld et al., 1995; Pennino et al., 1991). Supplementation for this nutrient is thus necessary. From a husbandry standpoint, the most worthy manner to provide supplementation is in such a way that all the food offered to an entire collection is supplemented in one concise undertaking; yet a uniform dispersal and consumption of the nutrients is still vital. The methods of supplementation can be divided into two categories: 1) those that enhance the nutrient contents of the prey item (ex: dusting, gut-loading and dipping) and 2) those which offer the nutrients directly to the animal being fed (ex: injectable, oral or topical-cutaneous dosings).

Although the level of additional vitamin A achieved in prey items after various supplementation methods has been evaluated, the ultimate effects on the vitamin A status of the target animal through a multi-month feeding period have not been measured.

Objective

The objective of this study was to evaluate the circulating blood plasma retinol levels of a captive adult population of endangered Puerto Rican crested toads (*Peltophryne lemur)* after prolonged periods of three differing vitamin A supplementation regimens. The appropriateness of each supplementation practice as compared to a control was determined.

Methods

All care, handling and experimental methodology was approved by the North Carolina Zoological Park's *Institutional Animal Care and Use Committee.* Puerto Rican crested toads housed at the North Carolina Zoological Park in Asheboro, NC, part of a non-releasable population, were used in this study. Forty-eight adult toads were blocked by weight into a control and 3 treatment groups in a randomized block design (three replicates per treatment, four toads per housing unit). For control: toads were fed a control diet without supplementation of vitamin A, Treatment 1: toads were fed additional vitamin A via dusting of crickets – Reptivite D_3, Treatment 2: toads were fed a control diet without vitamin A supplementation but receiving an oral dose of vitamin A (25 IU/g body weights [BW]) once per week (retinyl palmitate), and Treatment 3: toads were fed a diet of crickets gut-loaded with vitamin A (70: retinyl acetate/30: retinyl palmitate mixed in water and misted on cricket food). After 69 days of acclimation to the control diet and new housing conditions, all toads were placed on their respective treatments for 102 days. Cricket intake was measured three times per week and BW were measured at each blood collection (days 0, 46, 74 and 102, after the initial 69 day adaptation). Toads were bled through the ventral abdominal vein or by cardiocentesis and the blood was processed and stored until analysis. Blood plasma from assigned toad pairings within housing units was pooled prior to analysis. Retinol was measured in the plasma by reverse-phase high-performance liquid chromatography following procedures as outlined by Arnaud et al. (1991). Non-nutritional husbandry of toads (cleaning, temperature and lighting) continued as usual for the facility throughout the entire study period.

Results and Discussion

A BW range of 39.4 to 121.5 g (average = 76.1 g) was observed throughout the study. The BW remained constant through the trial for all treatments. At the end of the study, toads in treatment 1 had higher average circulating plasma retinol concentrations (298.4 ng/mL) than the control toads (31.5 ng/mL; $P < 0.0001$). Toad averages for treatments 2 and 3 did not differ from the control (6.6 and 0.0 ng/mL, respectively; $P > 0.0695$). It is believed that a depletion of vitamin A from body stores had started on d 0, after the 69 days without vitamin A supplementation as these levels were their lower than those observed to occur in treatments that responded. Toads on the control diet showed lowest levels of plasma retinol after 143 days without vitamin A (d 74; 23.95 ng/mL). Collectively, this study showed that providing vitamin A supplementation through the dusting of crickets may result in higher circulating retinol levels than gut-loading and oral supplementation.

Ensuring adequate provision of nutrients in diets is only one of many components which serve to assure the proper nutrition of species in captive settings. Evaluating whether a supplementation strategy has the desired impact on the animal being cared for is ultimately necessary to validate the methods used.

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DIET AND DIABETES IN PALLAS' CATS, *OTOCOLOBUS MANUL*

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Diabetes is a rare condition in wild felids, unlike in domestic cats. Recently, two Pallas' cats at different institutions were recognized as diabetic. Both were maintained on commercial wet and dry cat food. One was euthanized because of secondary complications, and the other has been managed for the past 18 months with a variety of strategies. This male Pallas' cat was 10 years old and asymptomatic when elevated serum glucose was detected during a routine physical examination. Initially, switching the Pallas' cat from commercial processed food to specific pathogen free (SPF) rodents corrected the hyperglycemia and glucosuria. After maintaining the Pallas' cat on rodents for 5 months, he was gradually switched back to commercial cat food over a 6-week period and developed glucosuria that again resolved after resuming a SPF rodent diet. The response to a diet change and his age supported a diagnosis of type 2 or non-insulin dependent diabetes. Eleven months after diagnosis, diet alone failed to control the elevated serum glucose and glucosuria. Oral glipizide was started at 0.25 mg/kg for one month and then increased to 0.50 mg/kg for an additional month. Glipizide stimulates increased secretion of insulin by the pancreas if functional beta cells are present, but liver damage is a potential side effect. There was no change in blood glucose and fructosamine levels during this 2-month period, but the glipizide was discontinued because of a 3-fold increase in the liver specific enzyme, alanine tramsaminase (ALT). Sixteen months after diagnosis, the Pallas' cat became symptomatic with polyuria and polydipsia. He is currently being managed with a single daily injection of insulin glargine (rDNA origin), a long acting basal insulin analogue, prior to being fed. The recommended dose of 0.5 units/kg has not reduced blood glucose and the dose is gradually being increased every 10-14 days. Urine is monitored daily for ketones for evidence of ketoacidosis. The goal is to determine a regimen that will reduce blood glucose with a single daily injection of insulin to demonstrate the feasibility of managing a diabetic wild felid.

LONGITUDINAL CHANGES IN THE COMPOSITION OF MILK FROM *GORILLA GORILLA*

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The lack of longitudinal milk composition data in nonhuman primates primarily is due to the fact that milk collection generally requires the mother to be separated from her offspring and anesthetized, both of which are minimized in the wild and captivity. Animal care staff at the Smithsonian National Zoological Park trained an adult female *Gorilla gorilla* to permit unsedated physical examination, including manual expression of the mammary glands. The relationship between this adult female and her care staff allowed milk samples to be collected weekly from the offspring's birth in January 2009 and samples continue to be collected. A subset of the samples collected between January 2009 and June 2010 have been analyzed for protein (n = 24), fat (n = 19), and sugar (n = 19). This subset included at least one sample from each month of lactation over that time period. The goals of this project are: 1) to document the pattern of compositional changes over the course of lactation in *G. gorilla*, and 2) to demonstrate that with training milk collection is possible from a wide range of species in a zoo setting with minimal invasive procedures. Protein content was higher and more variable in the first 45 days of lactation (1.94 ± 0.15% versus 1.0 ± 0.01% , $P<.001$). In samples after 45 days protein (minimum = 0.9%, maximum = 1.1%) and sugar (minimum = 6.7%, maximum = 7.8%) were relatively constant; fat was the most variable (minimum = 0.8%, maximum = 3.3%) and tended to increase with infant age ($r=.448$, $p=.071$). These compositional changes are similar to those seen in human milk. Higher protein concentration early in lactation may reflect increased immunoglobin content and increases in milk fat later in lactation may reflect inclusion of solid foods in the offspring's diet, and thus longer internursing intervals. Gorilla milk appears remarkably similar to human milk on these parameters.

TANA RIVER YELLOW BABOON (*PAPIO CYNOCEPHALUS*) DIETARY CHOICES: A MACRONUTRIENT COMPARISON OF FOREST VS. SAVANNAH FOODS

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Little has been reported regarding dietary preferences of the Tana River Primate National Reserve, Kenya, yellow baboons (*Papio cynocephalus*). Here, we present nutritional analyses of 116 flora specimens (56 species; 31 families) consumed by the Mchelelo baboon troop (N≈75 members), comparing forest to savannah samples. Consumption data represent 5 years of hourly scan-sample observations JAN88–OCT92 (N=55 months; 875 observation days; 4,893 hourly scans). Flora were collected MAR08–APR09, air dried, and shipped to the Smithsonian National Zoo Nutrition Lab where the first author determined macronutrient content via standard procedures: van Soest fiber (NDF/ADF), bomb calorimeter (gross energy - GE), ashing (minerals), CHN (crude protein - CP), and Soxhlet (lipids). We predicted higher mean GE for the forest flora due to a greater percentage being fruit/seeds and higher fiber content for the savannah flora due to a greater percentage being grass/corms. While the greater GE of forest samples was supported, the greater fiber of savannah samples was not. However, savannah flora had significantly higher CP, mineral/ash, and a CP/fiber ratio than did the forest samples. There was no significant difference in lipid content. Additionally, we found significant positive correlations between GE and lipids, NDF and ADF, and CP and mineral/ash content with significant negative correlations between NDF and lipids, and ADF and CP. Our data provide basic information on the nutritional choices of this particular baboon population and underscore two potentially important patterns relevant to understanding baboon behavior. First, they highlight the tremendous importance of the forest and its products to some populations of baboons – a pattern of habitat usage that may have far-reaching effects on other species and the habitat itself. Second, despite the noted differences in forest vs. savannah flora, we show similarity in macronutrients across samples that may point towards a baboon "ideal" food product.

MINERAL CONCENTRATIONS AND DART FROG CONSUMPTION RATES OF CULTIVATED FRUIT FLIES

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Fruit flies (*Drosophila sp*.) are the food choice for several captive species; however, little research has been conducted regarding their nutrient profile. The North Carolina Museum of Natural Sciences, Raleigh, NC houses several species of Dart Frogs (*Dendrobatidae* family) with the objective of maintaining breeding populations. These frog species have a carnivorous dietary strategy and because many known insects cultivated as food sources have an incomplete mineral profile (Ca in particular), it is vital to determine the nutrient content of the fruit flies used. Therefore, the mineral profile (Ca, P, K, Mg, Fe and Zn) for the two fruit flies species (*Drosophila hydei & Drosophila melanogaster*) fed at the museum were determined. The current study investigated the mineral profiles by collecting representative quantities of the fruit flies resulting in six mixed species samples of 0.5 g each. Three of these samples were dusted with a commercial mineral supplement (Rep-Cal Calcium with Vitamin D_3, Rep-Cal Research Labs, Los Gatos, CA) and three were not. All six samples of fruit flies were dried, ground into powder and then submitted for mineral analysis to the North Carolina State University Analytical Spectroscopy Service Laboratory using ICP (Inductively Coupled Plasma Mass Spectrometry). Dusted populations contained considerably higher concentrations of Ca, Mg and Fe (Table 1). When compared to published normal values for unsupplemented adult fruit flies (*D. melanogaster*) and crickets (*Acheta domestica*) (Bernard and Allen, 1997), the fruit flies in the current study contained higher concentrations of Ca, Mg and Fe (Table 1). The fruit flies that were not dusted (from the current study and previous literature) had lower concentrations of all minerals, except Fe than published results for crickets that had not been dusted. To better understand the total mineral consumption from these two fruit fly species, 19 frogs (6 *Phyllobates aurotaenia*; 4 *Dendrobates azureus*; 5 *Dendrobates leucomelas*; 2 *Dendrobates tinctorius* & 2 unknown) were monitored for 5 feeding days to determine actual fruit fly intake. The average daily fruit fly consumption per frog was 17.7 with a range of 5 to 55 flies per day. Clearly, dusting fruit flies successfully increased their Ca, Mg and Fe content. Future work is needed to 1) determine the dart frog mineral requirements and 2) determine the circulating mineral levels within the captive dart frogs to evaluate whether fruit flies dusted with a Ca supplement will in fact deliver the appropriate Ca levels needed.

Table 1: Dusted and Undusted Fruit Fly Mineral Concentrations (mg/kg) Compared to Published Adult Fruit Fly and Cricket Normal Values.

Mineral	P	Ca	K	Mg	Fe	Zn
			mg/kg			
Dusted	8755	22283	9353	4989	747	94
Not Dusted	9068	597	8948	1101	129	121
Pub Cricket[a]	9900	1400	12900	1300	58	188
Pub Fruit Fly[a]	10500	1000	10600	800	138	171

[a] Published values from Bernard and Allen, 1997

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NUTRITIONAL COMPOSITION OF ACTUAL AND POTENTIAL INSECT PREY FOR THE KASAKELA CHIMPANZEES OF GOMBE STREAM NATIONAL PARK, TANZANIA.

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Introduction

Populations of humans (Bodenheimer 1951, DeFoliart 1989), chimpanzees (Goodall 1986, Nishida 1973, Tutin and Fernandez 1992, Deblauwe and Janssens 2008), gorillas (Tutin and Fernandez 1992, Deblauwe and Janssens 2008) and orangutans (Fox *et al.* 2004) are known to regularly consume insects. Nonhuman apes most often prey upon eusocial insects such as termites, ants, and bees (McGrew 1992, McGrew 2001). Some insects can provide nutritional benefits comparable to meat on a gram-for-gram basis (DeFoliart 1989).

While edible insects are abundant in the tropics, patterns of insectivory are not universal across populations of any ape species. For example, populations of chimpanzees in the Mahale Mountains of Tanzania consume some species of insects that are ignored by those of Gombe (and vice-versa), though the communities are only 150km apart (Collins and McGrew 1987). The technologies employed to acquire insect prey vary between chimpanzee populations (McGrew 1992). Some of this variation is explainable by differences in the availability (Collins and McGrew 1987) or behavior (Schöning *et al*. 2008) of insect prey. Other variations lack robust ecological explanations and may reflect cultural differences between communities (McGrew 1992, Schöning *et al.* 2008).

Estimating the foraging returns for insectivory (McGrew 2001) requires nutritional data for the insects in question. Such data are also helpful for comparing insectivory patterns between populations (Hladik 1977) since even closely related insect species may have different nutritional compositions (Redford and Dorea 1984, Oyarzun *et al.* 2006)

Here we present the nutritional composition of insects consumed by the Kasakela chimpanzees of Gombe Stream National Park, Tanzania. We also present the nutritional composition of some insects that are abundant at Gombe and ignored by resident chimpanzees, but known to be consumed by apes elsewhere in Africa.

Methods

Insect samples were collected by RCO in 2008 over two field seasons (Feb-Apr and Aug-Dec). Drawing on nearly five decades of previous observations (Goodall 1986), we identified and collected insects that Kasakela chimpanzees were known to consume as well as other insects available and that were known to be consumed by apes elsewhere in Africa (McGrew 1992). Samples of all available castes of social insects were collected. Insects were weighed within 4 hours of collection to determine their fresh weight, desiccated in a drying oven at ~65ºC for 12 hours, and reweighed before storage in sealed glass vials.

Nutritional assays were conducted in the nutrition laboratory of the Department of Zoological Research at the National Zoological Park. Assays followed standard methodologies (AOAC 1990). Gross

energy (GE) was determined through bomb calorimetry. Ash (mineral) content was determined by combustion in an ashing furnace. Protein content was estimated through CHN analysis (using a standard conversion, %N x 6.25). Fat content was estimated using a micro-Soxhlet procedure. Fiber (chitin) content was estimated through the acid detergent fiber (ADF) procedure, with the resulting value corrected for ash content. In a few cases it was not possible to conduct all assays due to insufficient sample material.

Results

On a dry-matter basis insects consumed by Kasakela chimpanzees had significantly lower values for ash ($Z=-2.74$, $p=.0061$) and higher GE ($Z=2.62$, $p=.0088$) but were not significantly different in chitin ($Z=-0.18$, $p=.8572$), estimated fat ($Z=1.39$, $p=0.1645$) or protein ($Z=-0.03$, $p=.9767$) from the other insects assayed. When compared on a fresh-weight basis the insects consumed by Kasakela chimpanzees had significantly lower values for ash ($Z=-2.97$, $p=.003$) but did not differ in GE ($Z=0.95$, $p=.3421$), chitin ($Z=-0.21$, $p=.8337$), estimated fat ($Z=1.06$, $p=.2891$), or protein ($Z=-0.09$, $p=.9283$) from the other insects assayed (all tests are 2-tailed).

On both a fresh and dry matter basis, termite and ant alates were high in estimated fat, as were all castes of *Oecophylla longinoda*. Worker termites were high in ash (mineral) content relative to other castes. Major soldiers of *Pseudocanthotermes spiniger* had the highest estimated fat content of any termite soldier or worker.

Discussion

Of the available palatable insects, Kasakela chimpanzees prefer insect prey that are high in energy and low in ash. However, gross nutritional content is insufficient to explain prey choice. Kasakela chimpanzees ignore some prey (*Crematogaster spp., Camponotus maculatus* and *Pseudocanthotermes spiniger* soldiers) that are palatable, accessible and abundant in Gombe NP (O'Malley, unpublished data). Vitamin and mineral content were not assayed in this study but may also be important. Deblauwe and Janssens (2008) found that termite consumption by sympatric chimpanzees and gorillas resulted in high intake of manganese and iron, respectively, though it was not possible to determine if those nutrients drove prey choice.

Other likely influences on prey choice include the relative abundance and distribution of social insect nests across the community range, differences in potential intake rates of various species and castes, the nutritional returns for different insects on a per-unit (i.e. per-insect) basis, and socially learned preferences for certain prey over others. A broader analysis of the influence of these factors on insectivory by Kasakela chimpanzees is currently underway.

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