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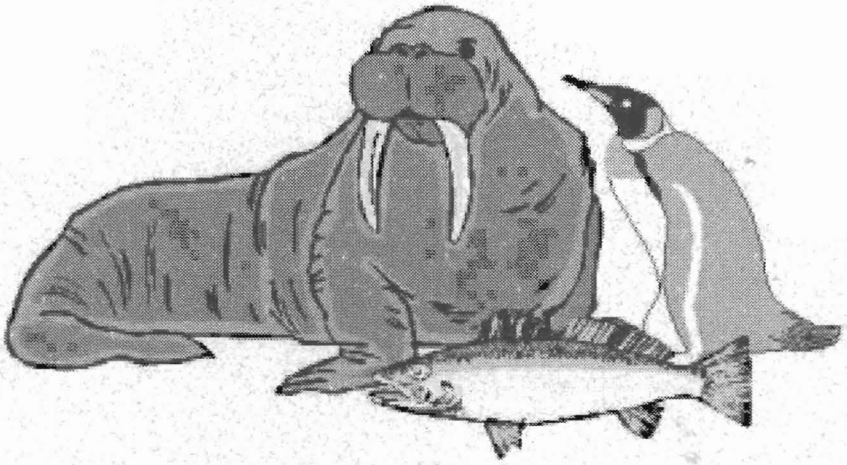
COLLEGE OF VETERINARY MEDICINE



Third Annual

Crissey Zoological Nutrition Symposium

Raleigh, North Carolina

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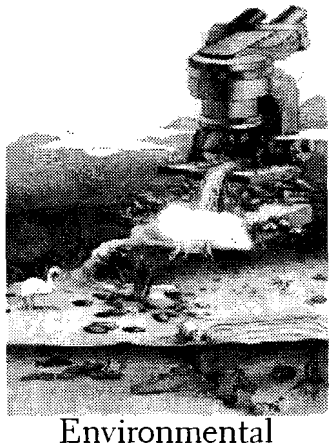

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The Crissey Zoological Nutrition Symposium is an Environmental Medicine Consortium Project supported by the generous contributions of donors to the Environmental Medicine Endowment of the North Carolina Veterinary Medical Foundation.

Second Annual Crissey Zoological Nutrition Symposium
Raleigh, North Carolina
December 10 and 11, 2004

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

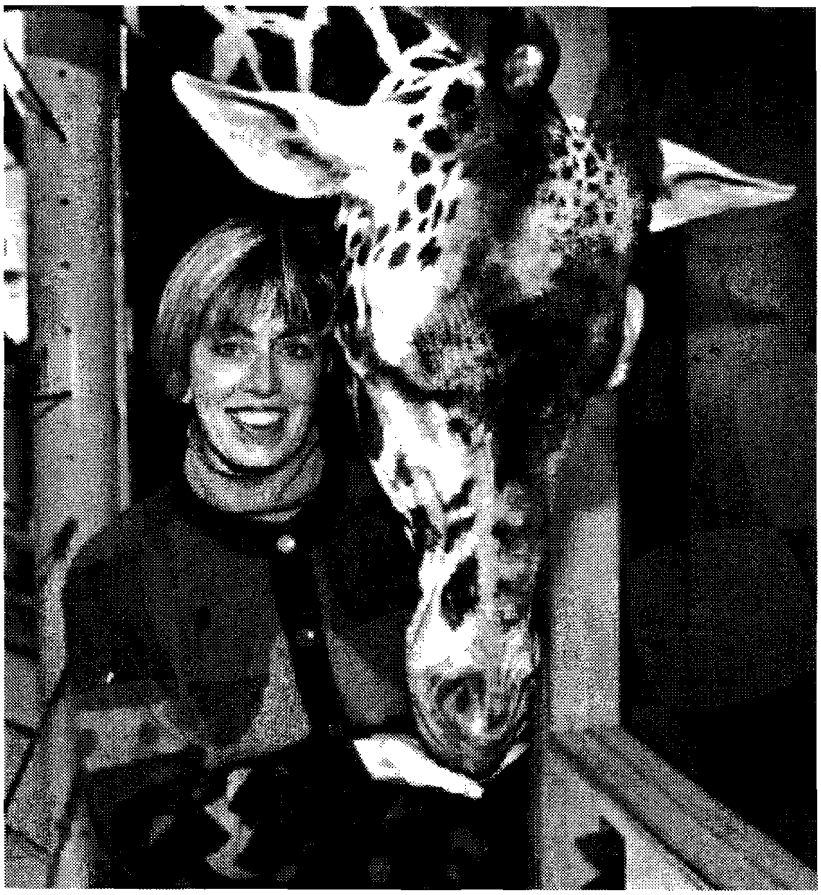
Sue Crissey earned her B.S. and M.S. degrees in human nutrition from Michigan State University and spent four years with the FDA before accepting a scholarship from the University of Maryland to pursue a Ph.D. in animal nutrition. She completed a post-doctoral fellowship at the Smithsonian Institution's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

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

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

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

- Sue Crissey, 2002



Susan D. Crissey

The Key Note Speaker

Ruth Francis-Floyd (DVM, MS, DACZM) is a veterinary specialist in aquatic animal medicine. She has a joint appointment with the Department of Fisheries and Aquatic Sciences and the Department of Large Animal Clinical Sciences at the University of Florida. Following completion of graduate school, Dr. Francis-Floyd worked in the channel catfish industry conducting research on *Edwardsiella ictaluri*, an important bacterial disease, at the Delta Branch Experiment Station in Stoneville, Mississippi. She has been with the University of Florida since 1987 and is the extension veterinarian for aquaculture. Her responsibilities include research and extension on health related issues for the ornamental fish industry, as well as coordination of diagnostic support services for aquaculture industries in the state. She also works closely with the Florida Game and Fresh Water Fish Commission on health concerns of Florida's freshwater sportfish.

Representative Publications of Interest

Francis-Floyd, R. 1999. Clinical evaluation of fish in private collections. Veterinary Clinics of North America: Exotic Animal Practice, W.B. Saunders Company, Philadelphia, PA 2:247-264.

Francis-Floyd, R. 1998. Drug availability for minor species in the 21st century: ornamental fish. Veterinary and Human Toxicology 40 (supplement): 25-27.

Francis-Floyd, P. Reed, P. Gibbs, E. Shotts,B. Bolon, W. Coleman, R. E. Klinger. 1998. Isolation of *Achòleplasma laidlawii*. from fish in a central Florida lake. Journal of Aquatic Animal Health. 10:252-258.

Francis-Floyd, R., Gildae, J., Reed, P, Klinger, R. 1997. Efficacy of Bayluscide (Bayer 73) for control of nuisance snails in ornamental fish ponds. Journal of Aquatic Animal Health 9:41-48.

Francis-Floyd, R., P. Reed, B. Bolon, J. Estes and S. McKinney. 1993. An epizootic of *Edwardsiella tarda* in largemouth bass (*Micropterus salmoides*). J. Wildlife Diseases 29(2):334-336.

Third Annual Crissey Zoological Nutrition Symposium
Schedule of Events and Table of Contents

Friday, December 9, 2005

12:30 – 1:00pm	Registration		
1:00 – 1:30pm	Conference Introduction & Welcome		
Session I: Nutrition of Aquatic Species I Session Chair: Kimberly Ange-van Heugten			
Time	**Author**	**Title**	**Abstract on Page**
1:30 – 1:45pm	Barry Nash, MS	Assessing Seafood Quality	11
1:45 – 2:00pm	Greg Bolton, MS	Development of Histamine in Scromboid Fish	13
2:00 – 2:15pm	Harry Daniels, PhD	Nutrition and Feeding of Southern Flounder, *Paralichthys lethostigma*	15
2:15 – 2:30pm	Allison Tuttle, DVM	Captive Shark Nutrition: The Big Black Box	17
2:30 – 2:45pm	Laura Osegueda	Information Strategies and Resources for Aquatic Animal Nutrition	19
2:45 – 3:00pm	Questions & Answer Session		
3:00 – 3:15pm	Break		
Session II: Nutrition of Aquatic Species II Session Chair: Dr. Michael Stoskopf			
3:15 – 3:30pm	Michael Stoskopf, DVM, PhD, DACZM	Condundrum I	21
3:30 – 3:45pm	Robert Browne, PhD	The Uncoupling of Protein Availability from Growth and Phenotypic Plasticity in Tadpoles	25
3:45 – 4:00pm	Anita Tucker, MSc	TSH (Thyroid Stimulating Hormone) Test For Evaluation Of Thyroid Function In Captive Trumpeter Swans (*Cygnus buccinator*)	29
4:00 – 4:15pm	Michelle Mehalick	Comparison of Currently Utilized Harbor Seal Hand-Rearing Formulas	31
4:15 – 4:30pm	Gary Lynch, PhD	Aquatic Nutrition	35
4:30 – 5:00pm	Question & Answer Session		
5:00 – 6:30pm	Reception – Hosted by Natural Balance		
6:30 – 7:30pm	Ruth Francis-Floyd, DVM, MS, DACZM	**Keynote Address: Lifestyles of Famous Fishes: Better Living through Natural History**	

Saturday, December 10, 2005

8:00 – 9:00am	Continental Breakfast		
Session III: Fiber, Texture, and Forages Session Chair: Dr. Michael Stoskopf			
Time	**Author**	**Title**	**Abstract on Page**
9:00 – 9:15am	Michael Stoskopf, DVM, PhD, DACZM	Conundrum II	37
9:15 – 9:30am	Martha Manning, BSc	Oral Stereotypies and Dietary Fibre in Captive Giraffes (*Giraffa cameloparadalis*)	39
9:30 – 9:45am	Suzanne Kennedy-Stoskopf, DVM, PHD, DACZM	Effect of Diet on Dental Scoring and Blood Parameters in Pallas' Cats (*Otocolobus manul*)	41
9:45 – 10:00am	Dan Johnson, DVM	Experience Using Critical Care™ and Carnivore Care™ with Wildlife and Zoo Species	43
10:00 – 10:15am	Mark Alley, DVM	Tall Fescue Pasture in Zoological Settings	45
10:15 – 10:30am	Question & Answer Session		
10:30 – 10:45am	Break		
Session IV: Physiology & Primate Nutrition Session Chair: Kimberly Ange-van Heugten			
10:45 – 11:00am	Kimberly Ange-van Heugten, MS, PAS	Conundrum III	47
11:00 – 11:15am	Esther Finegan, PhD	A Website Providing Illustrations to Support the Teaching of Comparative Physiology of Digestion and Nutrition	49
11:15 – 11:30am	Michael Power, PhD	Primate Milk in an Evolutionary Perspective	51
11:30 – 11:45am	Michael Jarcho	Investigating the Interplay among Digestion, Serum 25-hydroxyvitamin D and Bone Mineral in the Common Marmoset Monkey (*Callithrix jacchus*)	53
11:45am – Noon	Saskia Timmer	Survey of Spider and Woolly Monkey Diets and Health Histories at Thirteen Institutions	57
Noon – 12:15pm	Question & Answer Session		
12:15 – 1:30pm	Lunch		

Session V: Color, Constipation and Clinical Cases Session Chair: Kimberly Ange-van Heugten			
Time	**Author**	**Title**	**Abstract on Page**
1:30 – 1:45pm	Kimberly Ange-van Heugten, MS, PAS	Conundrum IV	61
1:45 – 2:00pm	Wendy Hood, PhD	Male Color, but not Body Composition, Reveals Food Deprivation in the American Goldfinch (*Carduelis tristis*)	63
2:00 – 2:15pm	Robert MacLean, DVM	Constipation in an Aging African Warthog (*Phacochoerus africanus*): A Case Report	65
2:15 – 2:30pm	Karen Wolf, MS, DVM	Passerine Rickets in a Zoological Aviary	69
2:30 – 2:45pm	Question & Answer Session		
2:45 – 3:00pm	Break		
Session VI: Analytical Nutrition Session Chair: Dr. Michael Stoskopf			
3:00 – 3:15pm	Michael Stoskopf, DVM, PhD, DACZM	Conundrum V	71
3:15 – 3:35pm	Roxane Fagan, PhD	Tracking Diets in Animals: A Stable Isotope Approach	73
3:35 – 4:00pm	Robert Browne, PhD	Omega-3 Fatty Acids and High Levels of Vitamin A Improve the Growth (Part 1*.), and the Health (Part 2#.) of the Endangered Wyoming Toad (*Bufo baxteri*)	75
4:00 – 4:15pm	Question & Answer Session		
4:15 – 4:30pm	Conference Closing Remarks		

ASSESSING SEAFOOD QUALITY

Barry Nash, MS
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The physical, chemical, nutritional, and bacteriological characteristics of fish vary with the species, season, diet, and harvest location. Nonetheless, it is possible to describe the changes that occur during decomposition after death, eventually leading to totally spoiled fish. The quality of seafood is dependent on how it is handled at the point of harvest, processing, storage and distribution. There is no single subjective or objective measure to ascertain the post-harvest quality of marine species. The typical metrics used by seafood technologists to measure product quality involve evaluating data from both qualitative and quantitative methods that target threshold indicators of sensory, protein, lipid, and microbiological degradation. Perhaps the easiest measure of quality is the visual examination. Whole and dressed fish, fish fillets, crustaceans, shellfish and frozen seafood exhibit specific visual characteristics that can clearly denote any abuse these products have incurred in the distribution system. Overall, the quality of seafood is maintained by keeping product moist and cold, limiting its exposure to atmospheric oxygen and minimizing temperature abuse. Handling measures that protect the quality of seafood also maintain its nutritive content. This presentation will discuss the quintessential indicators of physical quality and generally describe the handling procedures that protect the sensory, microbiological and nutritional characteristics of fresh seafood.

The quality characteristics of **cut fish** include firm elastic flesh, translucent color, moist appearance, and a mild, ocean-like aroma. Avoid cut fish having a soft flabby texture, bruised flesh, brown and / or dry edges, milky appearance, and an ammonia-like odor.

Whole and dressed fish should have bright, shiny eyes, bright-red gills, firm elastic flesh, scales that adhere tightly to the skin, smooth glistening skin, a mild ocean-like aroma, and a bright-pink belly cavity. Avoid product having cloudy sunken eyes, pale or gray gills, soft flabby flesh, dull and missing scales, excess surface slime, a sour ammonia-like odor, or a dark-brown blood streak in the gut cavity.

Frozen fish should be solidly frozen at receipt. Reject any product that shows evidence of excess ice crystals as temperature abuse may have occurred during transit. Once it has been thawed, frozen fish should be held to the same criteria as unfrozen, whole, or dressed fish. Frozen, cut fish should be packaged in low-moisture-permeable film to prevent the loss of surface moisture that leads to freezer burn.

Live shellfish should exhibit tightly closed shells; (if open, they shut when tapped), intact moist shells and a mild scent. One should avoid gaping shells that do not shut when tapped. Additional characteristics of inferior product include animals having cracked, chipped, or dry shells as well as those that emit a strong "fishy" odor.

Shucked oysters should be plump and free of shell and sand particles. The pack liquid should be clear and comprise less than 10% of the product volume, and the meat should have a mild, ocean-like scent. Avoid shriveled, dark, or dry-looking meat or product packed in cloudy or milky-looking liquid. Reject any product that emits a sour or "fishy" aroma.

Fresh scallops should have a sweet odor, be free of excess liquid, and should have a creamy white, light tan, or pale-pink appearance. High-quality **shrimp** should have translucent shells with grayish green, pinkish-tan or pink tint, moist appearance, firm flesh, and mild odor. Avoid shrimp with blackened edges or spots on the shell (except spot prawns), a reddish appearance and soft flesh.

DEVELOPMENT OF HISTAMINE IN SCOMBROID FISH

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Histamine poisoning is a chemical intoxication resulting from the ingestion of food that contains high levels of histamine and possibly other biogenic amines (putrecine, cadaverine, scombrotoxin, et al.). It is one of the three most common causes of food borne illnesses associated with the consumption of seafood in the U.S. Diagnosis of the illness is usually based on patient's symptoms, time of onset and the effect of treatment with antihistamine medication. The incubation time for the disease is short, usually a few minutes to a few hours, and initial symptoms can include tingling and a burning sensation around the mouth, an upper body rash, pruritis and hypotension. Symptoms can progress to nausea, vomiting and diarrhea and may require hospitalization, particularly for elderly or otherwise impaired patients. The disease is usually self-limiting but it can be life threatening. It is most often confused with food allergy.

Histamine is formed in food fish by the reaction of L-histidine and the enzyme histidine decarboxylase. Certain fish species have high levels of free histidine that can be released by proteolytic action (autolytic or bacterial) to be available for enzymatic conversion to histamine. These fish species include members of the Scombridae: tuna, mackerel, bonito; the Scomberesocidae: saury; and many nonscombroid fishes such as mahi-mahi, bluefish, jacks, yellowtail, herring, sardines, and anchovies. Strains of naturally occurring bacteria found in the environment and on the fish can produce histidine decarboxylase under ideal growth conditions further adding to the problem.

Formation of histamine in food fish occurs when scombroid or other high histidine content fish are not chilled properly after harvest allowing bacteria to proliferate and produce enzymes. The enzymes will then begin converting free histidine in the fish tissue into histamine and other compounds. Temperature abuse at any point from harvest to consumption can lead to histamine formation. Unfortunately, once histamine is formed there is no known practical way to remove it.

Organoleptic testing can fail to detect histamine toxic fish. The US Food and Drug Administration will not allow fish with histamine concentrations greater than 50ppm to be sold for human consumption. Dose response in humans can vary but 50ppm is considered hazardous. Species resistance to histamine can vary as it does among individuals, but similar caution (< 50 ppm) is appropriate in evaluation of food fish for picivorous animals. The control strategies for histamine contamination are simply time/temperature requirements. Internal fish temperatures must be obtained within a specified time after death. The temperature requirement is =40°F but the time varies by species, handling practices and environmental conditions at harvest.

Interestingly, pure histamine administered orally does not provoke adverse effect at high doses (150-200 mg) in most humans. This suggests that fish must contain some substances that

potentiate the toxicity of histamine. In vitro studies implicate other biogenic amines such as cadaverine and putrescine. These amines inhibit enzymes involved in detoxification of histamine. Histamine though used as the indicator of fish spoilage, has a heterogeneous distribution in and among fish, necessitating an intensive sampling scheme. Cadaverine is more uniformly distributed in spoiling fish, suggesting that testing for cadaverine may be a better index. Unfortunately cadaverine testing is cost and time prohibitive with currently available technology. ELISA based test kits for histamine are available that are reasonably quick and affordable.

Histamine Tests Kits

Neogen Corp. Alert® for Histamine (ELISA)
Screens samples at 50ppm
Approximately $5 per sample

Neogen Corp. Veratox for Histamine (ELISA)
Quantitative from 2.5 to 50 ppm
Approximately $6 per sample

Testing Labs (GC or HPLC)
Southern Testing & Research Laboratories, Wilson NC
Pert Laboratories, Edenton, NC
Silliker Laboratories, Homewood, IL

NUTRITION AND FEEDING OF SOUTHERN FLOUNDER, *PARALICHTHYS LETHOSTIGMA*

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Southern flounder, *Paralichthys lethostigma*, are left-eyed flounders belonging to the family Paralichthidae. During the past decade, interest in the use of southern flounder for aquaculture and stock enhancement has spurred the development of improved culture techniques. The complex life cycle of flounder demands different feeding strategies for each life stage. These differences in feeding behavior must be recognized and understood to ensure optimum growth and survival

Larval flounder require small (50-250 micron), slow-swimming, live feed (rotifers and *Artemia*). Failure to properly enrich live feed with docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) at this stage is a major cause of poor survival and abnormal pigmentation.

Postmetamorphic flounder are easily weaned from live feed to dry, commercial diets. Overcrowding at this stage leads to excessive cannibalism, low survival, and growth stunting of a portion of the population. Conversely, low stocking densities cause reduced feeding activity, weight loss and eventual starvation. Behavioral changes caused by culture techniques have a major impact on the nutrition and feeding of flounder and influence population growth and sexual dimorphism.

Female broodstock are asynchronous spawners capable of maintaining egg production over several months under controlled photothermal conditions. Nutritional requirements of broodstock are not well defined, especially at they relate to the effects of diet formulations on egg quality. Based on their spawning characteristics and sensitivity to photothermal cues, we hypothesize that a research strategy to study broodstock nutrition should be modeled after the approaches used in the poultry industry, instead of the ones used to study synchronous-spawning fish such as rainbow trout (*Oncorhynchus mykiss*).

CAPTIVE SHARK NUTRITION: THE BIG BLACK BOX

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Sharks are a major display species for many aquariums worldwide. They are popular, expensive, and difficult to obtain and/or replace. They are analogous to marine mammals in this sense and, as such, should be maintained with the same high level of care required for captive marine mammals. Reported health problems in captive sharks have included obesity, wasting, excessively fatty livers, goiter, buoyancy problems, scoliosis, dermal plaques, parasites, trauma, and complications associated with stomach eversions. While there may be no clear etiology established for all of these problems, it is possible to invoke a nutritional component in the pathogenesis of each of these problems. Unfortunately nutritional problems have not been well-investigated in captive sharks.

A standard approach to deciding what to feed a captive shark should involve a determination of what the species feeds upon in the wild. The available published literature describing the gastrointestinal contents for several species of wild-caught sharks confirms that not all sharks feed equally. Some species (ex. sandtiger, lemon, blacktip, and hammerhead sharks) appear to feed mainly on other fish while other species (ex. nurse and bonnethead sharks) appear to eat a diet consisting of mixed invertebrates. Further, some species (ex. sandbar sharks) eat a mixed diet of fish and invertebrates. As such, it makes sense that captive sharks would be fed to approximate what they eat in the wild instead of adopting a single feeding protocol for all species. Then at the next level of refinement, it makes sense to take into account body condition, appetite, activity level, age, and reproductive status when determining how much and how often to feed.

A small scale survey of shark feeding practices in display aquariums confirmed a diversity of approaches to shark nutrition. Six US aquariums were surveyed on what shark species were kept and, for each shark species, what was fed, how much and how often, as well as what was supplemented, and the rationale for the feeding protocol. Differences in food species fed, feeding schedule and vitamin supplementation suggest there is a possibility for future studies to improve captive shark nutrition. A more sound scientific basis for shark nutrition programs would be clinically valuable and scientific research in this area should be encouraged.

Vitamin supplementation for captive sharks is a common practice regardless of the composition of their diet. Unfortunately, little is known about the vitamin and mineral needs of these animals and what pathology results from deficiencies or excesses of these vitamins and minerals. The two most commonly used products used to supplement shark diets, Mazuri's Vita-Zu Sharks/Rays vitamin and SeaTabs for Birds, Turtles, Fish and Sharks, are dramatically different in composition (Table 1). The Mazuri supplement contains calcium which is not included SeaTabs while the SeaTabs product contains folic acid, biotin, choline, inositol, iron, copper, magnesium, zinc, manganese, and kelp which are not found in the Mazuri supplement.

This variability raises the question of which level of what supplement is appropriate to maintain the health of various species of sharks in captivity.

TABLE 1. Guaranteed Analysis of specific vitamins and Iodine in Mazuri Vita Zu Sharks/ Rays Vitamin (Mazuri) and Sea Tabs for Birds, Turtles, fish and sharks (Sea Tabs) standardized to the recommended dose per kg of food.

Nutrient	Mazuri	SeaTabs
Vitamin A	11,000 IU	2,200 IU
Vitamin B1 (Thiamine)	165 mg	110 mg
Vitamin B2 (Riboflavin)	3.3 mg	0.55 mg
Vitamin B3 (Niacin)	0.013 mg	0.32 mg
Vitamin B5 (Pantothenic Acid)	8.2 mg	3.3 mg
Vitamin B6 (Pyridoxine)	2.2 mg	0.33 mg
Vitamin B12 (Cyancobalamin)	8.2 mcg	4.4 mcg
Vitamin C	1100 mg	22 mg
Vitamin D3	1375 IU	44 IU
Vitamin E	110 IU	110 IU
Iodine	550 mg	0.015 mg

It is our hope to encourage a careful examination and improvement of the husbandry and health management practices for captive sharks. Much needed information on captive shark nutrition is lacking and that we encourage scientific investigation into the nutritional requirements of the commonly captive held species. In addition, we recommend the practice of conducting individual annual physical examinations including weight and girth measurements and the careful and consistent recording of food delivery combined with at least proximate analysis of the food items being feed to captive sharks.

INFORMATION RESOURCES AND SEARCH STRATEGIES FOR AQUATIC NUTRITION

Laura M. Osegueda
NC State College of Veterinary Medicine
Veterinary Medical Library

Veterinary and wildlife biologists use the internet everyday for communication and information updates from news resources and colleagues. Learn how to set up automatic search updates on PubMed and CABI. Take advantage of new individualized search options in bibliographic databases and internet websites. Take a few searching tips from your friendly librarian in how to track down just the right research articles.

WHAT IS A CONUNDRUM?

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

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

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

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

In addition, we would like to solicit conundra form the symposium participants for discussion next year. Interesting questions, contradicting dogmas, are all welcome as possible conundra. Submit your ideas by e-mail to Kimberly Ange-van Heugten (kimberly_ange@ncsu.edu) or Michael Stoskopf (michael_stoskopf@ncsu.edu) as they come to mind over the next year.

CONUNDRUM I

Recommended maximum storage times for frozen food fish vary significantly with the lipid content and composition of the fish species, and frequently don't correspond with the intervals between catch seasons. For example, appropriate sized herring may be fished for only for a few weeks out of the year, yet recommended storage times wouldn't allow a facility to hold relatively fatty herring for an entire year. Of course the degradation of frozen fish isn't an instant event, and some degradation occurs throughout the storage.

What is the best way to deal with the challenge presented by limited fishing seasons and short recommended storage times?

Are there practical ways to assess fish quality if recommended fish holding times are being exceeded?

Should we be concerned about degradation earlier in the storage period, and if so, what and how?

THE UNCOUPLING OF PROTEIN AVAILABILITY FROM GROWTH AND PHENOTYPIC PLASTICITY IN TADPOLES

Robert K Browne* PhD[1,2,3], Melissa Pomering MS[3],
Andrew J Hamer PhD[3], Micheal Mahony PhD[3].
Address: [1] Department of Biology, University of Memphis, Memphis, TN 18152, USA; [2] Memphis Zoo, 2000 Prentiss Place, Memphis, TN 38112,USA:[3] Department of Biological Sciences, University of Newcastle, University Drive, Callaghan NSW 2308, Australia.

Introduction

The efficient production of high quality anuran (frogs and toad) metamorphs is increasingly required in conservation programs, and for their commercial production for consumption, display, or as pets. Anuran larvae are unique among vertebrates for their phenotypic plasticity; 1) of morphometrics (including the easily assessed tail length:snout-vent length) and 2) in development rates. The Australian green and golden bell frog's (*Litoria aurea's*) life stage characteristics share many ecological and growth characteristics with Ranids and other large anurans, which are threatened and commonly used for consumption. The tadpoles of *L. aurea* grow to a large weight at metamorphosis and in nature are generalists that filter feed or actively browse.

Past studies in the field and *in vitro* have shown that anuran's metamorph weight and metamorphosis time are highly variable. In the field reduced metamorph weight and shortened metamorphosis time have been shown in habitats of short duration. Similarly *in vitro* studies comparing stable water levels with drying tanks have shown an average reduction in metamorph weight and shortened metamorphosis time in drying tanks, and that within a treatment that later metamorphs are generally larger. Other studies have shown increased tadpole density lowers metamorphosis weight and increases metamorphosis time. The proximate mechanisms for these various phenotypic plasticities have been considered as responses of the endocrine system to increased levels of corticosteroids. Few studies have considered nutrient availability as affecting phenotypic plasticity. However, in the commercial production of metamorphs for consumption, phenotypic plasticity has been attributed to both exploitative and interference competition for food. The quality and availability of nutrients also could be limited in many *in vitro* studies where the most common sources of nutrients are lettuce, or boiled lettuce, or these sometimes supplemented with fish flakes. The differences in nutrient density between tanks with stable water levels and those drying could also confound phenotypic responses, when both treatments receive the same amount of food. To uncouple the effect of nutrition on phenotypic plasticity in tadpoles we studied the affect on metamorph weight, metamorphosis time, and morphometrics, of ad libartum feeding of tadpoles with high protein feed.

Experiments

In Experiment 1, *L. aurea* tadpoles were reared in shallow trays (1.5 L) at high densities (40, 80, 120, and 180 L^{-1}) and ad libartum amounts of high protein feed. Metamorphosis commenced at 42 days post-hatch and the experiment was terminated at 100 days post-hatch. Survival was high in all treatments. The mean metamorphosis time (front leg emergence, Gosner stage 42), and weight (Wt; 0.1 g); and snout-vent, tail, and total length (TL) were measured (0.1 mm). Condition index (CI) was calculated CI = (Wt g / TL^3 mm) x 10^4, and also the ratio of tail length:snout-vent length. Where needed a correlation was calculated to interpolate projected early juvenile weight after tail absorption by metamorphosing tadpoles from the total length at stage 42. In Experiment 2, tadpoles of frog species from ephemeral (n=3) and permanent (n=3) ponds were raised at a density of 60 L^{-1} using the protocol above and their early juvenile weight and metamorphosis time measured.

Results and discussion

In Experiment 1, the tail length/snout-vent length ratio remained constant in the 60 L^{-1} density over the metamorphosis period, but increased in the other treatments. This morphometric stability suggests that at the 60 L^{-1} density there were limited density effects on phenotypic plasticity. The weight at metamorphosis in all treatments declined with increasing metamorphosis time from 4.2 ± 0.1 to 1.7 ± 0.2 g, with the greatest decline within a treatment in tadpoles at 60 L^{-1}. The reasons for this are obscure as the amount of food per. tadpole increased as metamorphs were removed. In many previous *in vitro* studies metamorph weight increased with metamorphosis time, suggesting that limitations in food reduced the weight of early metamorphs. As metamorphosis time increased the condition index declined in tadpoles at 60 and 80 L^{-1} but increased in the densities of 120 and 180 L^{-1}. This decline in the treatments of higher density could indicate a shortage of food for the earlier metamorphs. From 40 - 80 L^{-1} time to metamorphosis was 54 - 56 days, far less than the 80-100 days in other studies; and the early juvenile weight of 2.10 -1.90 g was greater than that of those in nature (1.8 - 0.9 g). These differences indicate that in previous studies of *L. aurea* that nutrient limitation could have a major affect on the expression of phenotypic plasticity (some data in Table 1).

Table 1. Correlations between initial density and metamorphosis Wt and CI in *Litoria aurea* calculated from replicate means at 4 day intervals from 45 - 85 days. An exponential decay function ($y = y_0 + ae^{-bx}$) provided the best fit to data. The maximum (Pr max) and minimum (Pr min) predicted (by the exponential decay function) metamorphosis weight and condition index from 45 - 85 days. Pr max = predicted maximum weight, Pr min = predicted minimum weight.

Correlations between days post hatch (DPH) and metamorphosis weight							
Treatment	**Prob>|t|**	**R^2**	**y_0**	**a**	**b**	**Pr max (g)**	**Pr min (g)**
60	0.005	0.69	1.79	5729	0.1685	3.58	1.80
120	0.0001	0.85	1.88	1299	0.1380	3.60	1.89
180	0.0092	0.46	1.96	165.8	0.1106	2.79	1.98
240	0.0002	0.64	1.83	83.4	0.1001	2.51	1.84

In Experiment 2 there was no relationship between metamorphosis weight and metamorphosis time, showing that early juvenile weight is independent of time when the effects of nutrient availability are negated. Metamorphosis time clustered at 23-27 d in species from ephemeral ponds, and extended from 45 d in those from more permanent ponds indicting an inherent metamorphosis time depending on habitat (Table 2).

Table 2. The mean time (days) to late metamorphois (stage 46, Gosner, 1962) metamorph and mass (g) and egg weight of four myobatrachids and three hylid species (n=2 trays). Lim. or., *Limnodynastes ornatus*; Lech. fl., *Lechriotus fletcheri*; Lit. chl., *Litoria chloris*; Lit. les., *Litoria leseuri*; Lim. tas., *Limnodynastes tasmaniensis*; Ad. br., *Adelotus brevis*; Lit. au., *Litoria aurea*. Habitat type; #short-term, ^impermanent, * stream. Means ± SE.

Species	**# Lit. chl.**	**# Lech fl.**	**# Lim. or.**	**^Ad. br.**	**^Lim. tas.**	***Lit. les.**	**^Lit. au.**
Metamorphosis time	23.4 ± 0.8	25 ± 0.2	24 ± 3.7	45 ± 0.3	47 ± 3.0	48 ± 2.0	53 ± 0.4
Weight	0.77 ± 0.08	0.20 ± 0.04	0.40 ± 0.11	0.17 ± 0.2	0.51 ± 0.04	0.51 ± 0.13	2.1 ± 0.69

Conclusions

The results of this study indicate that limited nutrition can have a major influence on the results of studies of tadpole phenoplasticity. Consequently, many studies could benefit form a control using the high nutrition protocol if used as a baseline for phenotypic responses to environmental limitations. *Litoria aurea* tadpoles share many ecological and growth traits with commercial species, and this protocol may enable increases in rearing density coupled with increased metamorph weight and lowered metamorphosis time.

TSH (THYROID STIMULATING HORMONE) TEST FOR EVALUATION OF THYROID FUNCTION IN CAPTIVE TRUMPETER SWANS (*CYGNUS BUCCINATOR*)

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A captive flock of trumpeter swans held at Fair Lake, ON Canada had significant mortality during the winter of 2004 with inconclusive post-mortem results. Abnormal thyroid morphology indicated a possibility of thyroid disease (inherent or acquired), which may have resulted from either genetic predisposition or nutritional deficiency. In addition, high liver iron was noted, possibly indicating exposure to excessively high levels of this mineral. Due to the population status of this species in Ontario and the importance of maintaining a healthy captive population prior to release, it was thought that thyroid function should be tested. The hormones produced by the thyroid, thyroxine (T_4) and triiodothyronine (T_3), are necessary for maintaining most major physiological functions, including basal metabolic rate & thermogenesis, fat, carbohydrate, and protein metabolism, cardiovascular function, bone turnover, molt, mental development, and normal growth & reproduction.

Thyroid function can be tested with a simple T_4 blood test if standard baseline values are known, however, no values have ever been reported for trumpeter swans. In addition, T_4 values can be influenced by stress, fever, systemic illness, drugs, varying environmental temperatures and energy balance. However, the T_4 response following a TSH injection will not be influenced by these factors, thus making it the best available test of thyroid function. The test has been used to successfully identify hypothyroidism in various species of psittacines, passerines, gulls, poultry, geese and pigeons. The objective of this study was to first determine 'normal' baseline T_4 values for this species, and subsequently evaluate hormone synthesis capabilities of the thyroid gland to determine if primary hypothyroidism is present in this captive flock of trumpeter swans.

Blood samples from twelve yearling trumpeter swans from a captive population were collected to determine baseline T_4 values. Immediately following this, an intramuscular injection of bovine TSH was given at a dosage of 0.30 mg/kg with the assumption that all birds weighed approximately 17 lbs (7.72 kg). Blood was collected again at 4 and 24 hours post-TSH injection. A significant T_4 increase was seen after 4 hours for 10 of the 12 swans ($P<0.001$). After 24 hours, 8 of the 10 birds continued to have rising T_4 values, while 2 had values which returned to baseline. Two of the 12 swans had no significant T_4 response to the TSH injection. These results indicate that two birds have a hypothyroid disorder that originates in the thyroid gland itself, and are thus incapable of producing a detectable thyroid hormone response. Further clinical examination of these birds is needed to determine the type of hypothyroidism present: infection, disease, chronic autoimmune thyroiditis, primary iodine deficiency, or ingestion of antithyroid agents. Since all birds in the flock had access to the same food and water, the latter two factors are not thought to be involved.

Liver samples from swans which died during the winter of 2004 at Fair Lake, ON were also evaluated for heavy metals. Only iron was found to be significantly higher than values previously reported in the literature ($P=0.01$). Significant Spearman's correlations were found

between chromium and cadmium ($P<0.05$), iron and zinc ($P<0.01$), iron and lead ($P<0.01$), and zinc and lead ($P<0.05$). Sediment and water samples from the enclosures were also analysed for heavy metals. No values were above those listed by the Canadian Environmental Quality Guidelines (2003). These findings appear to rule out overt mineral toxicity as a contributing factor in mortality of the flock.

COMPARISON OF CURRENTLY UTILIZED HARBOR SEAL HAND-REARING FORMULAS

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A number of facilities throughout the world routinely hand-rear neonatal harbor seal *(Phoca vitulina)* pups, yet there remains very little published data on the ingredients, cost and efficiency of the hand-rearing formulas that are utilized. Most of the information concerning hand-rearing formulas is relayed through personal communications between facilities, which prevents objective evaluations. As there is much variation in the budget, staffing and space availability in between facilities, a number of non-nutritional factors may influence the choice of formula ingredients. The primary goals of this study were to identify and document recipes that are currently and successfully used to hand-rear neonatal harbor seals and objectively compare all recipes by the cost per liter, availability of ingredients, preparation time, storage requirements, ease of preparation and final consistency. This format allows reasonable comparisons between recipes with respect to the variables that an individual facility finds significant to their circumstances. In addition, the content of fat, protein, moisture, fiber, and ash was determined for each formula. Because these formulas are currently and successfully used we infer that all recipes are nutritionally adequate. However, several of the participating facilities were able to provide data from pups receiving their formula, and thus a rough evaluation of nutritional efficiency could be constructed.

A project summary and request for recipes was created to which eight facilities responded. Two facilities submitted two recipes each and a final recipe was obtained from the 8th Ed. of the Merck Veterinary Manual for a total of eleven recipes. Four facilities responded to the additional questionnaire concerning data from animals receiving the hand-rearing formula that was submitted. Cost analysis was determined by an averaged cost of each ingredient obtained through on-line searches or contact with suppliers and the quantity of the ingredient per liter of formula. Cost of shipping was included, when applicable. Availability was assessed by assigning each ingredient a score from 1 to 5 on the basis of specific criteria, which were then averaged to obtain a single score for each recipe. Storage requirements were determined similarly. Ease and length of preparation time and formula consistency was assessed by randomly preparing each recipe 5 times: twice using a hand-mixing method, twice using a blender, and a 5th time doubling the volume, using the mixing method indicated by the instructions. Single batch recipes were adjusted so that total volume was between 600 and 900 mL. The consistency of the formula prepared in each of the single batch trials was assessed immediately after preparation and after 1 and 24 hours of refrigeration at 4° C. Formulas prepared during the double batch trials were drawn through an 18-french red rubber catheter immediately after preparation and after refrigeration and re-heating to room temperature. A numerical score for consistency was assigned based on these observations. Average preparation time of single batches was used for time comparisons and included the measurement of ingredients. The ease of preparation was

evaluated by the lengthiness of the preparation instructions with respect to their necessity to obtain the desired product and from which were assigned numerical scores. Three 50 mL volumes of each formula prepared in the second blender method trial were obtained immediately after completion and placed in a -20° C (and later -80° C) freezer and were shortly submitted to an outside laboratory for proximate analysis of fat, protein, moisture, ash and fiber content. Nutritional competency of the formulas in pups was quantified using an equation for 'conversion efficiency', defined as the pup's weight gain in kilograms over days 1 through 20 divided by the total volume of formula fed from days 1 through 19. Additionally, facilities were asked to rate the frequency of which they observed clinical signs of poor digestion in animals receiving the formula and any other occurrences that they judged to be significant. We requested that the pup data be from animals less than 5 days old upon their arrival, that they survived until weaning and that there was no indication of severe trauma or disease.

Recipes showed wide variation in percentage of ingredients but all utilized either herring (n=4), Zoologic Milk Matrix 30/55® (n=4) or a combination of the two (n=3) as the primary ingredient(s). Other ingredients included water, all recipes, fish oil (n=8) and heavy whipping cream (n=3). Supplements such as lecithin and multivitamins were a common addition. The number of ingredients per recipe ranged from 2 to 11. Cost range of formulas was found to be $0.22- $6.49 per liter (mean= $3.74) and was determined mainly by the inclusion of milk matrix, the mean cost of milk matrix (without herring) recipes more than twice that of those containing herring only. The number of ingredients was not shown to influence the cost but did lengthen preparation time, as did the complexity of the recipe and the presence of fish. The mixing method was not shown to influence preparation time and the blender mixing trials of most formulas were slightly longer than the hand-mixing trials. There was a significant difference in consistency scoring between recipes but all formulas returned to original consistency after re-warming and brief hand mixing and were drawn thought the catheter with little difficulty. The availability and storage requirements of varied little between recipes, where all scores fell within a 1.8 and 1.0 range of a 5-point scale, respectively.

For comparison of nutritional content recipes were assigned to mainly herring (n=7) or mainly milk matrix (n=4) categories by weight of the aforementioned ingredients. Milk matrix recipes had much higher fat and caloric content while herring recipes contained more moisture. The average protein content was higher in herring recipes, but not statistically significant.

Pup data was obtained from 4 facilities. The plethora of variables involved with this data does not allow confident conclusions to be drawn but potentially offers some insight on the individual formulas and does present some intriguing contrasts that may aid in the design of further studies. Of the data received, two recipes used milk matrix and no herring, one used herring and no milk matrix, and the final a combination of the two, but mainly herring. One of each ingredient category was a facility that received the *P. v. concolor* sub-species, the other receiving the *P.v. richardsi* sub-species. The conversion efficiency of the *concolor* pups was much higher for the mainly herring recipe in contrast to the *richardsi* pups, which showed equally greater conversion efficiency with the milk matrix only recipe. Studies in free-ranging animals show a lower daily weight gain and longer lactation in the *richardsi* animals, potentially suggesting a difference in milk composition, but more noteworthy as a consideration when assessing the efficacy of a hand-rearing formula. Pups receiving the herring recipes weaned in

nearly half the time of those fed the milk matrix recipes. This combined with the higher cost of milk matrix formulas remarkably increases the total cost per animal. Both facilities using milk matrix based recipes reported vomiting in their pups and less commonly, constipation and poor attitude. These signs were not reported from facilities using herring based recipes.

AQUATIC NUTRITION

Gary Lynch, Ph.D.
Mazuri®

Due to both their digestive physiology and the impact their environment, the nutritional requirements of a fish and aquatic feeding programs are quite unique in comparison to other animals. In general, the actual nutrient requirements of fish are similar to a typical monogastric animal. Physiologically, fish have a short and simple digestive tract compared to terrestrial animals. A fishes G.I. tract will typically be 1-1.5 times its body length. This compares to a 4:1 G.I. tract length to body length ratio for cats, and a 4:1 ratio compared to pigs. And fish generally have no saculation in their tract: Just a simple stomach and intestine. Thus there is no foregut fermentation and little to no hindgut fermentation. And with few exceptions, there is no mastication/grinding that takes place within the G.I tract.

Another physiological aspect that plays a fished nutrient digestion is its cold-blooded nature. The lower temperature in the G.I tract reduces the efficacy of digestive enzymes. Cold water fish have very little amylase and carbohydrate enzyme activity, which is why they tend to be carnivores. Their proteases and lipase are more effective at the lower temperatures, and typically use protein to meet their energy requirements. Warm water fish tend to be omnivorous. They have some amylase activity, and can utilize some carbohydrates. Carbohydrate utilization can be greatly improved when a cooked/gelatinized starch source is fed.

For all of these reasons, commercial diets tend to be composed of very highly digestible ingredients. This yields feed conversion ratios in the rang of 1:1 to 1.5:1 for fish, compared to 2:1 for commercial poultry, 3:1 for growing swine, and 5-7:1 for cattle. Also contributing to a fish's high feed conversion efficiency is that being cold-blooded water-based animals, they do not need to expend energy for thermal regulation, and their neutral buoyancy in water negates an energy requirement in respect to gravity. Fish also are very efficient in their excretion the excess nitrogen they consume. Rather than taking the energy to form urea, they can directly excrete NH_4 through their gills into the water.

Fish also have a couple of nutrient requirements that are unique compared to terrestrial animals. Due to no fore- or hindgut fermentation, fish cannot synthesize ascorbic acid, and thus it must be included in their diet. Certain cold water fish also have a requirement for omega-3 fatty acids. This is typically met by including fish meal or fish proteins in their diet.

CONUNDRUM II

There are many holes in our understanding of the dietary requirements of fishes other than production species. Many species do not approach their maximum life span in captivity. As the value of these fishes increases, their clinical management is becoming more sophisticated, and for some very valuable species, annual physical examinations are not out of the question.

What clinical indices might be appropriate to incorporate into fish physical examinations to begin to gather data for assessing the appropriateness of diets being provided?

Remember the challenge of handling fish out of water without jeopardizing their health.

ORAL STEREOTYPIES AND DIETARY FIBRE IN CAPTIVE GIRAFFES (*GIRAFFA CAMELOPARDALIS*)

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Repetitive, invariable behavioural patterns with no clear purpose are known as stereotypies, and have been associated with sub-optimal welfare in captive animals. Modern zoos aim to increase the welfare and thus conservational and educational value of their animals. The challenge of keeping non-domesticated animals in captivity can be addressed in part by investigating stereotypic behaviours in these animals. Captive giraffes, *Giraffa camelopardalis*, are known to perform oral stereotypies (such as licking, tongue-playing and chewing their enclosure) which have, in the past, been connected with feeding them an unnatural, quickly-consumed low-fibre diet. Ethograms, focusing on the time spent on feeding, ruminating and performing oral stereotypies, by one male and one female Masai giraffe housed at the Toronto Zoo were constructed. The ethograms are based on continuous observational techniques for 3 hours a day (between 0730h-0900h and again from 1030h-1200h) both prior to and subsequent to the provision of additional daily dietary fibre in the form of low-quality timothy hay (Neutral Detergent Fibre 51.8% dry matter, Acid Detergent Fibre 41.3% dry matter). The provision of additional dietary fibre was associated with a decrease in time spent performing oral stereotypies, and an increase in time spent ruminating, during the two observation periods (0730h -0900h, 1030h-1200h). This decrease in time spent performing oral stereotypies may be associated with the increased wellbeing of these animals.

EFFECT OF DIET FORM ON DENTAL SCORING AND BLOOD PARAMETERS IN PALLAS' CATS (*OTOCOLOBUS MANUL*)

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The Pallas' cat (*Otocolobus manul*) is a small-sized felid (4-6 kg, average body mass) that is threatened with extinction in its natural habitat in central Asia (Russia, Mongolia). Captive breeding programs have been established for this species under the guidance of the American Zoo and Aquarium Association's Species Survival Plan; and a research colony of specific pathogen free cats has been initiated at the North Carolina State University (NCSU) College of Veterinary Medicine in an effort to reduce the significant mortalities from toxoplasmosis, experienced by both neonatal and adult Pallas cats in captivity. In the wild, Pallas' cats rarely encounter *Toxoplasma* in their natural diet but, in captivity, exposure to this parasite occurs commonly through raw meat diets as well as rodents and birds captured in zoo exhibits. To eliminate potential exposure to *Toxoplasma*, it has been recommended that zoos house Pallas' cats in indoor exhibits and provide animals with a processed, *Toxoplasma*-free diet, as their sole food source. Effects of dietary nutrient concentration, as well as physical form, were investigated.

A collaborative study funded by the Iams Company was begun in November 2003 to evaluate nutritional health and reproductive differences that may be attributable to diet in Pallas cats. Diet treatments included: a) n=5 (3.2) cats at North Carolina State University's colony who had been reared on a raw horsemeat-based diet (Dallas Crown frozen meat feline diet) and, after 10 mo, switched to a mixed diet of canned beef and dry diet, to duplicate that consumed by b) the Cincinnati zoo colony, comprising n=6 cats (3.3) raised on a mixture of Iams moist (canned beef formula) and dry (Eukanuba Chicken and Rice) diets. Blood samples were collected at beginning of the trial, and repeated at 4- month intervals for CBC, serum chemistries, vitamins A, D, and E, taurine, elemental concentrations (Ca, P, Fe, Na, K, Se, Co, Cu, Mg, Zn), and serum fatty acid components. Urine samples collected at the same intervals were taken for pH, mineral, and creatinine assays. Digestibility of various diets was assessed using chromic oxide markers. Reproductive parameters were evaluated with semen collected during the breeding season (Feb-April), and assessed for sperm concentration, % motility, % abnormal sperm, and total volume. In addition, sperm fatty acid constituents were analyzed.

At the initiation of the trial period, a physical examination was performed including the tooth and palatine areas, and teeth were cleaned. Oral health was evaluated after 6 mo consuming wet diets, including scoring of gingivitis, plaque, and calculus condition, and teeth were

recleaned. Cats were switched to the mixed diets with dry food included, and the final oral evaluations took 4 to 6 mo later. Throughout the trials, NCSU cats were also fed whole SPF mice (~25 g) 4 times per week.

Depending on age, cats at the Cincinnati Zoo consumed 85 to 200 g of canned kitten or adult beef diet daily, mixed with ~60 g Eukanuba dry Chicken and Rice adult diet, hence the proportion of dry to wet diet varied from 23 to 41% of total as-fed weight consumed daily (proportions were greater in smaller, growing cats). NCSU cats consumed diets comprising 142 to 200 g Dallas Crown feline diet initially, then gradually transitioned onto diets containing up to 25% dry food.

Even though whole mice were fed throughout and contributed abrasives and enrichment to the NCSU colony cats, dental scoring suggested an effect of prepared diet form on calculus formation (NCSU average calculus score (± SD) 6.06 ± 2.20 associated with wet only diet versus 2.99 ± 2.41 (n=4 cats) following the mixed dry diet feeding). No differences were obvious in gingivitis or plaque scores due to either facility or diet examined. Feline Odonticlastic Resorptive Lesions were noted in 3 of 4 cats examined at the Cincinnati Zoo but not in the NCSU colony – the significance of this observation is not known at this time.

Hematologic values for captive Pallas cats did not differ significantly by diet or facility, although there was a trend for a lower white blood cell count in cats fed the commercial diet when paired comparisons were made at NCSU. Serum biochemistry data, however, differed substantially by diet for some parameters and, in some cases, appeared outside normal feline ranges. SGPT/ALT values, for example, were higher in the Cincinnati cats compared to NCSU (124.7 ± 56.6 vs. 53.7 ± 17.8, respectively; laboratory reference range 10 to 80). On the other hand, glucose values were higher for cats in the NCSU colony (overall average 171.2 ± 17.4 mg%) compared to Cincinnati zoo cats (132.7 ± 54.0 mg%), although tended to be lower on the meat-based diet compared to the commercial mixture within that colony, hence other effects apart from diet must be considered contributory. Of particular concern, the LDH values for cats fed the Dallas Crown meat diet were substantially elevated (339.8 ± 107.8 mU/ml) compared with means when fed the mixed moist/diet (255.5 ± 1422.8 mU/ml) for 4 to 6 mo. Cincinnati cats, raised exclusively on the commercial products, averaged circulating LDH concentrations of 136.8 ± 41.7 mU/ml; laboratory reference range 20-225). This observation may have both direct (lipid metabolism, body condition) as well as indirect (immune response, reproductive output) health impacts that warrant further investigation. Dietary effects may be subtle, and clinical imbalances may only manifest during stressful situations

The short-term duration of this study, small numbers of animals, and (to date), limited analytical values preclude wide interpretation of data. Nonetheless, this study provides baseline information that can be compared with samples taken from free-ranging Pallas cats, and animals held under other environmental and husbandry conditions, to improve our knowledge and management of this species.

EXPERIENCE USING CRITICAL CARE™ AND CARNIVORE CARE™ WITH WILDLIFE AND ZOO SPECIES

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Two commercially prepared syringe-feeding formulas (Critical Care™ and Carnivore Care™, Oxbow Pet Products, Murdock, NE) have gained wide acceptance in avian and exotic animal practice; however, both of these supportive care formulas have many potential applications in other fields of zoological medicine.

Critical Care is a complete, high-fiber, timothy hay-based formulation. The dry product is mixed with water to the desired consistency and then administered by syringe or feeding tube. Although Critical Care was originally designed for small herbivores such as rabbits, guinea pigs, chinchillas and prairie dogs, it has also been used successfully in many other herbivorous species, including degus, wallabies, cottontails, jackrabbits, deer, llamas, iguanas, tortoises, geese, and swans.

Carnivore Care is a highly digestible, high-energy, egg, chicken, and pork protein-based formulation designed for convalescing ferrets. The powdered formula mixes with water to a smooth consistency that readily flows through a syringe or feeding tube. While Carnivore Care is a complete diet for ferrets, it will also meet the nutrient requirements for other obligate carnivores and has been used successfully in dogs, cats, exotic felines, fennec foxes, raccoons, coatimundis, raptors, snakes, carnivorous lizards, and other species.

Critical Care and Carnivore Care have been used successfully in combination to provide nutritional support for omnivorous species such as hedgehogs, backyard poultry, peafowl, ducks, and koi. The powdered formulas have a long shelf life and can be prepared with a minimum of waste. In the future, Critical Care and Carnivore Care will likely prove as invaluable for the treatment of wildlife and zoo species as they have for the treatment of avian and exotic pets.

TALL FESCUE PASTURE IN ZOOLOGICAL SETTINGS

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Tall fescue and perennial ryegrass are cool season grasses commonly used as forage for livestock in temperate regions of the world. In many geographic locations, these grasses have a symbiotic relationship with fungal endophytes, in particular, the genus *Neotyphodium*. The grasses benefit by increased growth and better survival due to enhanced tolerance to such abiotic factors as drought and high temperatures. The infected plants out compete uninfected grasses, thereby insuring the survival of the fungal endophyte. Such mutualism, in turn, benefits pasture management because of more abundant biomass contributing to greater average daily gains (ADG) in grazing animals and less labor because of lower pasture maintenance.

A variety of chemical compounds have been described from endophyte-infected fescue, including phenols, lysergic acid amides and ergot alkaloids. The most abundant ergot alkaloid, ergovaline, has historically been implicated as the toxin causing fescue toxicosis in livestock. Ruminants grazing endophyte-infected fescue pastures may experience lowered ADG, increased body temperatures, rough haircoats and reduced conception rates. Mares will exhibit increased gestation lengths, agalactia, foal mortality and thickened placentas. Despite the recognition that endophyte-infected fescue is associated with animal production problems, it is still widely used for pasture in the southeastern United States.

The African Plains, an approximately 30 acre exhibit at the North Carolina Zoological Park, occupies what was formerly a dairy cow farm. The pasture is long-standing fescue. The exhibit currently houses a variety of artiodactylids, including impala, Thompson's gazelle, Defassa waterbuck and Nile lechwe. There has been no direct evidence of fescue toxicosis in this exhibit historically, although there has been concern that it could play a role in the health of these animals.

A recent survey was conducted to determine the prevalence of endophytes in the exhibit. The pasture was sub-divided into 7 sections and sampled according to the North Carolina Department of Agriculture's guidelines. A total of 30 tillers were collected from each section. Tall fescue is a clump grass and a tiller represents one stem with its roots from that clump. Each tiller is placed in a plastic bag and kept refrigerated until it can be sent to the state lab for histochemical processing. An aniline blue dye is used to detect the presence of endophytic fungal hyphae. Percent infestation ranged from 63-100%.

CONUNDRUM III

How can pasture choices impact conservation programs for high profile herbivores and what can be done to optimize the chances of reproductive success and health?

For the purposes of this conundrum consider the following scenario in the South Eastern United States.

A multi-million dollar renovation of an African elephant and white rhinoceros exhibit is being planned to focus on breeding programs for both species. The white rhinoceros are currently on Bermuda grass, but as part of the renovation, their exhibit will be shifted to a larger venue which is currently planted in decade or longer established fescue. (Hint for the taxonomically challenged: Rhinoceros are in the same order as horses, Perissodactyla.

What recommendations would you make to optimize the success of the white rhino breeding program?

ELEPHANTS SIRENIANS
HUMANTEES

A WEBSITE PROVIDING ILLUTRATIONS TO SUPPORT THE TEACHING OF COMPARATIVE PHYSIOLOGY, DIGESTION AND NUTRITION

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A website is being developed to make available the diagrams, tables and photographs from the CD (C. E. Stevens, 2001), text (C. E. Stevens & I. D. Hume, 1995), and lectures on the vertebrate digestive system. Many of the diagrams, tables and photographs are already used in teaching. This website will make these graphic images more freely and easily available for download and provide a useful resource to a wider audience. The current development, design, and format of the new website will be presented and discussed, and examples of the web pages shown.

75 Photos 250 Fig & Tables

Resolution

PRIMATE MILK IN AN EVOLUTIONARY PERSPECTIVE

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Lactation is a defining characteristic of mammals that is sadly understudied. Although all mammals lactate, there are a remarkable number of lactation strategies that have evolved. The lactation strategy of a species is the integration of the duration of lactation, the frequency of suckling, the volume of milk the mother produces per day, and the composition of that milk. Primates in general have relatively long lactations, nurse their infants frequently, and produce large quantities of dilute milk.

Within this general pattern there is variation among species that exhibits phylogentic patterns. For example, human milk is much more similar to chimpanzee milk than it is to baboon or macaque milk. Several lemur species from Madagascar produce relatively similar and invariant milks. It appears impossible to distinguish between the milks of different females, or even between species. Other primate species appear to produce more variable milks. There is convincing evidence in the rhesus macaque and the common marmoset that female condition has a significant effect on milk composition. Even in these variable milks, however, there appear to be aspects of constancy, which may reflect phylogentic constraints. Human milk may exhibit some unique characteristics. For example, human milk has very high concentrations of secretory IgA (SIgA); the level of SIgA in mid lactation human milk exceeds that measured for colostrum of rhesus macaques.

The Nutrition Laboratory at SNZP is engaged in a number of ongoing collaborations investigating the diversity of milk composition patterns in primates. In this presentation we will discuss some of the findings regarding the proximate composition of primate milk, and possible phylogenetic patterns.

Methods

Proximate analysis of milks was performed at the Nutrition Laboratory at the National Zoological Park, Washington DC. Dry matter was determined by oven drying 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. Total sugar is assayed by the phenol-sulfuric acid method, using lactose monohydrate as the standard [Dubois et al., 1956; Marier & Boulet, 1959], with the results expressed on an anhydrous lactose basis. Crude protein is estimated from total nitrogen (TN) in the sample. Nitrogen is assayed using a CHN elemental gas analyzer (Model 2400, Perkin-Elmer, Norwalk, CT), which provides a rapid and accurate method of assaying TN in 20 µl milk samples. Crude protein (CP) is estimated as 6.38 x total nitrogen. Minerals are assayed after first digesting a subsample of the milk in nitric and perchloric acids. Minerals except for phosphorus are determined by atomic absorption spectrophotometry. Phosphorus is determined using ultraviolet spectroscospy.

There are data from the Nutrition Laboratory in the published literature on milk composition for a number of prosimian primate species (Tilden & Oftedal, 1997), and callitrichid monkeys from the New World (Power et al., 2002). There are data for a number of

cercopithicine species (e.g. macaques and baboons) in the published literature as well (reviewed in Oftedal & Iverson, 1995). In this talk we will present new data from great apes (bonobo, chimpanzee, gorilla and orangutan) and hylobatids (gibbon and siamang).

Results and Discussion

At the writing of this abstract all assays have not yet been completed. However, dry matter, fat and sugar concentrations of ape milks are similar to those found in human milk. There appears to be little variation within and among species. Apes (including humans) appear to produce some of the more dilute and low energy milks among primates. Interestingly, the composition of ape milk is more similar to that of lemurs than it is to that of macaques. This is certainly not due to phylogeny.

In general, primate milk is relatively dilute and low energy. There are some exceptions; the lorisoids produce milks of moderately high fat content. Within the anthropoid primates mean values for fat content of milks range from 1 – 5%. However, in some species (e.g. macaques and marmosets) fat content can be extremely variable, ranging from less than 1% to over 13%.

An accurate characterization of the phylogenetic patterns of milk composition among primates still eludes us, but progress is being made, especially among the anthropoids. Even among anthropoids, however, there is considerable work to be done. For example, data on the composition of colobine milk is scarce. Thus an entire radiation of old world monkeys currently is unrepresented.

There are many other milk constituents of interest besides fat, crude protein, total sugar, and minerals. Oligosaccharides represent a significant part of the sugar fraction. Their biological function is uncertain. Interestingly, human milk has both a high concentration and high diversity of oligosaccharides. Long chain poly unsaturated fatty acids (LCPUFAs) are thought to be important for brain growth and development. There has been speculation in the anthropological literature that human milk might have higher concentrations of LCPUFAs than milks of other primates because of our extended period of post partum brain growth (Martin, 1996). We are currently examining this hypothesis.

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INVESTIGATING THE INTERPLAY AMONG DIGESTION, SERUM 25-HYDROXYVITAMIN D AND BONE MINERAL IN THE COMMON MARMOSET MONKEY

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Captive common marmosets (*Callithrix jacchus*) are infamous for experiencing serious digestive tract inflammation, typically taking the form of chronic lymphocytic enteritis (CLE). This syndrome leads to chronic and recurrent diarrhea and to malabsorption of nutrients. The term "marmoset wasting syndrome" refers to cases in which gut inflammation becomes so dramatic that the animal is unable to maintain body weight, resulting in eventual starvation and death (Layne and Power, 2003; Ludlage and Mansfield, 2003). A second common ailment of captive marmoset populations is metabolic bone disease. Bone mineral density is strongly influenced by vitamin D status. The biologically active form of vitamin D (1,25-dihydroxyvitamin D) regulates osteoblast and osteoclast activity, and enhances calcium absorption in the small intestine and decreases calcium excretion by the kidney (Holick, 2003; Schulkin, 2001). Malabsorption decreases the ability to obtain vitamin D, calcium and other nutrients, through the digestive tract. For most captive marmosets, diet is the only means of obtaining vitamin D, because they do not have exposure to sunlight. The main circulating form of vitamin D is 25-hydroxyvitamin D (25-OH-D), which is the best marker of vitamin D status (Holick, 2003).

We propose that many cases of metabolic bone disease in marmosets are the result of chronic, subclinical CLE. The proposed sequence is as follows: (1) some initial stimulus causes inflammation of the digestive tract resulting in reduced ability to absorb nutrients (2) reduced ability to vitamin D and calcium results in negative calcium balance (3) osteoclasts are activated to free up calcium from the skeleton in order to compensate for the deficiency (4) if the inflammation progresses to the "wasting" stage then the animal dies of general malnutrition, otherwise eventually enough calcium is extracted from the skeleton that bones become brittle and mobility of the animal is hindered.

Methods

Subjects: The animals used in this study were 24 singly housed adult common marmosets (10 female, 14 male) housed at the Southwest National Primate Research Center (SNPRC; San Antonio, TX). Animals ranged in age from 1.6 – 9.3 years, and in size from 227-474g. Common marmosets reach sexual maturity at 1.5 years, and reach their full adult size by three years of age.

Diet: Animals were fed a single-item purified agar-gelled diet twice daily. This is the base diet for the entire colony at SNPRC, and all subjects had been fed it since weaning.

Digestion trial protocol: Two four-day digestion trials were conducted in consecutive weeks. All diet offered was weighed prior to feeding, and all refusals were collected and weighed. A fresh food sample was reserved for nutritional analysis from each feeding period.

Excreted fecal was collected twice daily and weighed immediately prior to freezing. All food and fecal samples were frozen until assayed for dry matter, energy, protein and minerals.

Bone mineral density: Subjects were anaesthetized with ketamine and butorphanol, weighed (fasted weight) and scanned with dual-energy x-ray absorptiometry (DEXA).

Blood sampling: After DEXA analysis subjects were immediately bled (under anesthesia) yielding approximately 1 ml. Blood samples were spun down to extract serum for clinical blood chemistry, and an aliquot of each sample was reserved to 25-hydroxyvitamin D at the Wisconsin National Primate Research Center.

Nutritional analyses: Nutritional assays were completed at the Nutrition Laboratory of the Smithsonian's National Zoological Park (NZP; Washington, DC). Samples were oven dried at 100°C, and then weighed to determine dry matter. Energy was determined using an adiabatic bomb calorimeter. Nitrogen was determined using an elemental analyzer. Crude protein was calculated as nitrogen X 6.38. Calcium content was analyzed on an atomic absorption spectrometer and phosphorous on an ultraviolet spectrometer.

Initial Results

There was substantial variation in apparent digestibility among individuals (mean = 85.4±0.8%; range, 75.5% - 90.1%). There was a significant correlation between apparent dry matter digestibility and 25-OH-D ($r = 0.522$, $p = 0.11$), however the relationship was not a simple linear one. There was a strong relationship between these variables in animals with 25-OH-D < 100 ng/ml ($r = 0.766$, $p = 0.01$), and no apparent relationship between the variables for animals with 25-OH-D > 100ng/ml. Animals with poor vitamin D status (25-OH-D < 40 ng/ml; Power et al., 1997) had lower apparent digestibilities, serum Ca, serum P, and bone mineral content (BMC). Three individuals with ADDM below 81% had undetectable levels of 25-OH-D and dramatically reduced BMC. Results on the absorption of Ca and P from the diet are pending.

Discussion

Among apparently healthy adult common marmoset there was substantial variation in the ability to digest a purified diet, 25-OH-D status, serum Ca and P, and bone mineral content. The relationships among these variable appears complex, but certain animals display a syndrome of low apparent digestibility, low 25-OH-D status, and low bone mineral content. The pending data on Ca and P absorption may shed further light on the functional relationships among digestion, vitamin D status and bone mineral.

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SURVEY OF SPIDER AND WOOLLY MONKEY DIETS AND HEALTH HISTORIES AT THIRTEEN INSTITUTIONS

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Introduction

According to the last count of the International Species Information Systems data there are 68 captive woolly monkeys (*Lagothrix lagothricha sp.*) in zoos worldwide (www.ISIS.org, 2005). The maintenance and breeding of this species in captivity is not very successful (Mooney and Lee, 1999) and the number of individuals in the wild is considered vulnerable to extinction. A breeding program across zoos exists but it is in danger because of the shrinking number of captive animals. Numerous zoos have attempted to house successful breeding populations. Most of these zoos have not been successful in breeding this specie because of the poor reproductive performance and high infant mortality rates (Giddens, 1987; Miller et al., 1995). A healthy pregnancy is difficult to sustain because females typically decrease in body mass during pregnancy and have high rates of spontaneous abortions (Mooney and Lee, 1999). The health complications in these animals are similar to those of diabetes mellitus and hypertension in humans (Giddens, 1987) and are more pronounced during pregnancy. Arteriosclerotic changes in the placenta have been found, which may lead to placental insufficiency with severe consequences for the fetus (Debyser, 1995). In addition, genetic sensitivity to stress may also be a contributing factor to the shrinking numbers of woolly monkeys in captivity (Debyser, 1995).

In contrast to the situation with woolly monkeys, their closest living relative, the spider monkey (*Ateles sp.*), is maintained successfully in captivity (Nowak, 1999). Therefore, a survey was distributed to numerous zoological institutions to gather information regarding the captive diet and general health of woolly monkeys and spider monkeys. Data from this survey will be analyzed to elucidate possible causative factors to the problems that differentiate these two primate species.

Data Collection Method and Results

A survey was distributed to sixteen zoological and private institutions holding spider or woolly monkey species. Thirteen institutions responded to at least some portion of the survey. Two zoos no longer held the specie. This survey inquired into the current number, the daily diet content and feeding strategy, the housing and the social relationships of the captive monkeys. The survey also included questions regarding the reproductive success and general health status of the monkeys over the last fifteen years. Finally, the survey asked about each institution's willingness to cooperate in further research involving collection of feces and saliva for cortisol

measurements.

The 13 institutions held 60 spider monkeys (19 males, 41 females) and 28 woolly monkeys (15 males, 13 females). Of the 60 spider monkeys, 9 monkeys were continuously housed indoor only, while 51 were housed in a combination of indoor and outdoor enclosures depending on season and animal preference. Of the 28 woolly monkeys, three were continuously housed indoor only, while 25 were housed in a combination of indoor and outdoor depending on season and animal preference. The size of the housing and its specifics varied widely both among and within institutions in terms of enrichment items and measurements.

The diet offered and consumed varied greatly in components both within and among holding institutions. The majority of daily diets for both species met estimated nutrient requirements for new world monkeys with a few exceptions (NRC, 2003). Nearly all monkeys were fed twice daily but two woolly monkey institutions reportedly fed three to four times per day.

For the institutions (n=10) that reported health information there were 36 spider monkey births and 29 deaths and 32 woolly monkey births and 61 deaths during the last 15 years. Therefore, there were almost twice as many woolly monkeys deaths as births in contrast to the higher number of births than deaths for the institutions housing spider monkeys.

Twelve of the thirteen institutions that completed the survey were willing to continue this research project by allowing the researchers to collect fecal or saliva samples for cortisol analyses in order to elucidate dietary stress among species. Cortisol measurements as an indicator of diet and stress related problems have not been published in woolly monkeys although they have been conducted in human and in numerous non-human primates (Fuchs et al., 1997; Schapiro et al., 1993). The survey concepts that may elucidate potential increased cortisol measures include animal rank, housing, diet nutrients, food availability and the effort needed to acquire it (Abbot et al., 2003).

Continuing effort

Two European zoos have currently participated in the survey and we expect more surveys from this region within the next few months. Once all survey collections are complete statistical analyses will be conducted to isolate potential stressors and their solutions.

Acknowledgments

Gratitude is given to the zoological institutions and private collectors that contributed to the survey. Contributors included: Apenheul Primate Park, Barranquilla Foundation, Gladys Porter Zoo, Fundacion Zoologica de Cali, Omaha's Henry Doorly Zoo, Little Mans Zoo, Little Rock Zoological Gardens, Louisville Zoological Garden, Quinta de Santo Inacio, The Rendall's and Yokohama Zoological Gardens. A special thank you is extended to Mrs. Karen Caster, Dr. Jenifer Chatfield, Dr. Cheryl Dikeman, and Pat & Herlar Faircloth for their research assistance and hospitality.

The authors also are extremely grateful to Mazuri (PMI Nutrition International) for the partial funding of this research. Without their support this project simply would not have been possible.

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CONUNDRUM IV

An important form of data gathering for studying the efficacy of captive diets is the survey of institutions that are successfully holding the species of interest. It seems like a fairly simple process, but it is common for the data being returned from surveys to be of little use to the investigator rather than the boon to understanding expected. If surveys are too long, they aren't completed and submitted. If they are not specific enough, a number of very major variables may be hidden behind a "too broad" query. Questions are sometimes unintentionally ambiguous, and answers may not reliably depict the true situation.

What methods can be used in surveying zoos and aquaria for nutritional data, to optimize the usability of the data collected?

How can you optimize compliance with the survey request?

How can you validate the data being returned from an extensive survey?

MALE COLOR, BUT NOT BODY COMPOSITION, REVEALS FOOD DEPRIVATION IN THE AMERICAN GOLDFINCH

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Ornamental traits, such as the conspicuous colors displayed in many male birds, are often used by females to gain specific information about the quality of prospective mates. A female bird that chooses a male with maximum color expression may gain resources and/or good-genes benefits by mating with highly ornamented males. The red and yellow color displays in the feathers of songbirds are among the best studied ornamental traits. These color displays are the result of carotenoid pigmentation, and birds must ingest intact carotenoid pigments and move them to growing feathers to produce red and yellow coloration. Studies on two species of cardueline finches, the House Finch (*Carpodacus mexicanus*) and American Goldfinch (*Carduelis tristis*), have examined the independent effects of environmental challenges during molt and found that access to carotenoid pigments, food access, and parasite load can each have a significant effect on production of male carotenoid coloration. At the same time, these variables have been found not to affect black melanin coloration of feathers. Although color expression in wild birds is undoubtedly associated with the cumulative and interactive effects of carotenoid intake, foraging success, and parasite load, no prior studies have examined these factors simultaneously nor has it been determined if food restriction impacts body composition. Poor body condition may negatively impact physiological mechanisms responsible carotenoids deposition, but the mechanism by which food deprivation during molt affects carotenoid feather coloration is unstudied. In this study, we simultaneously manipulated carotenoid intake, food access, and parasite load of captive American Goldfinches during the molt of yellow body feathers and black crown feathers. At the end of the experiment we quantified the color quality of yellow body feathers, the color quality of black crown feathers, and the size of the black cap. We also measured the effect of the treatment on total body water, total body fat, and total lean mass to determine if food restriction impacts body composition.

Male American Goldfinches were collected from large winter flocks prior to the prealternate molt in February 2005. Animals were housed in an aviary at Auburn University in groups of two in small (0.5 m^3) cages. Animals were assigned to one of 8 treatment groups with either high or low carotenoid access, high or low food access, and high or low parasites. High and low carotenoid levels were assigned based on previous aviary studies and analyses of wild birds to approximate high and low intake among wild birds. High-food-access birds had ad lib food access; low-access birds had food removed from caged for 6-hr blocks of time totaling about 38% of daylight hours. Low-parasite birds were treated with an anti-coccidial drug; high-parasite birds were untreated and developed coccidiosis. After holding birds under these conditions through prealternate (spring) molt, animals were killed. The light reflectance from feathers was measured using a spectrometer, and software was used to calculate hue, chroma, and brightness of yellow carotenoid coloration and brightness and contrast of black crown feathers. Crown patch size was estimated as crown length measured with a caliper. Whole animal homogenates were dried to constant mass and fat was extracted from duplicate subsamples with petroleum ether in a Soxhlet apparatus. From these data, we calculated percent dry mass, percent

fat as a function of dry mass percent lean dry mass of the animals. We used a factorial ANOVA to look at the relative effects of pigment access, food access, and parasites expression of plumage coloration and body composition.

Carotenoid access had a large effect on the hue and yellow chroma of breast feathers. There was also a significant interaction affect of parasites and carotenoid access on the hue of yellow feathers. Food access did not affect the hue or yellow chroma of feathers but there was a significant interaction effect of carotenoid access and food access on UV chroma of yellow feathers. Food access had a significant effect on the UV contrast of black crown feathers. There was no treatment effect on the size of black crown patches. Despite the effect of food access on UV chroma of yellow feathers and UV contrast of black feathers, there was no significant effect of restricted access to food on body composition, specifically total dry mass, total body fat as a function of dry mass, and total lean mass as a function of dry mass.

These observations generally corroborate previous, single-variable studies showing effects of carotenoid access, food access, and parasites on carotenoid coloration. Our observations, however, highlight the importance of a multifactorial experimental design as many of the strongest effects on color display came through the interaction of pigment access, food, and parasites. Consistent with several recent studies, we found an effect of food access on the color quality of black melanin pigmentation, but no effect of any environmental variable on the size of the black badge produced. Of particular interest is our observation that animals given limited access to food did not differ in body composition relative to those fed *ad lib*, yet food access had significant effects of color expression. The significance of these observations for the information content and likely function of these color displays will be discussed.

CONSTIPATION IN AN AGING AFRICAN WARTHOG (*PHACOCHOERUS AFRICANUS*): A CASE REPORT

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This report describes an episode of severe constipation in an intact 14-year-old 100 kg female African warthog (*Phacochoerus africanus*) at the North Carolina Zoological Park that resolved with both medical and surgical therapies. This animal was previously diagnosed with carpal osteoarthritis and was on oral glucosamine therapy. Scant dry feces were reported periodically starting in June, 2004, and these episodes were treated successfully with 8 oz canned pumpkin b.i.d. Her daily diet, divided into two feedings, consisted of 0.5 lb apples, 1.25 lb carrots, 0.5 lb corn 3d/wk, 0.25 lb sweet potato 4d/wk, 1.3 lb Mazuri Herbivore pellets, ½ flake timothy hay, ½ flake alfalfa hay, and water ad libitum.

On June 19th, 2005 (day 1), the warthog was reluctant to stand and had not defecated for two days. Three days of anti-inflammatory therapy was initiated and ½ cup pumpkin was added to her diet. She had a normal bowel movement and was standing again on day two. On day five, however, she was anorexic and had not had another bowel movement. The keepers noted that fruit from several recently-matured mock orange trees of the genus *Poncirus* was dropping into the warthog exhibit, but they were unsure whether the animal had consumed it. *Poncirus* spp. fruit is a gastrointestinal saponin glycoside toxin that can cause discomfort and constipation that is usually temporary. Saponins may cause foaming around ingesta and decrease the efficacy of peristalsis.

With no improvement on day six, the animal was immobilized for examination with 250 mg xylazine, 35 mg butorphanol, and 25 mg midazolam i.m. The caudal abdomen was moderately distended with gas and five firm, adhered fecal balls were manually removed with the aid of enemas; 80 ml mineral oil was also gavaged. With no bowel movements, worsening lethargy, and increasing abdominal distention by day 8, the animal was again immobilized, and radiographs revealed gas-filled, moderately distended loops of bowel. Due to concerns of a possible toxic etiology, a surgical ventral laparotomy was elected. A fist-sized firm fecal mass, consisting of alfalfa-like stems, but no other plant material, was removed from the centrifugal colon. The orad and caudad ingesta was loose with fluid. A pedunculated, multilobulated, spongy purple mass, measuring 10 x 5 x 3 cm, was emanating from the distal jejunum with no signs of adjacent intestinal strangulation. Histopathology reported the mass as a benign mesenchymal tumor. Other intestines and organs appeared normal. The animal was recovered and remained in the hospital. Pain was managed with 15 mg butorphanol i.m., b.i.d. and an anti-inflammatory, and parenteral antibiotics were initiated.

After 3 days of anorexia and lethargy, the animal ate 2 cups of grain and fruit mash. The next morning, day 12, the animal presented with projectile vomiting. Due to concerns of peritonitis and dehydration, the animal was again immobilized. Copious firm feces were palpable, and abdominal radiographs showed moderate gas distention and a ground glass

appearance suspicious for peritonitis. The abdominal exploration was repeated and a large fecal mass with orad gas accumulation was removed from the centripetal colon. The entire caudad colon was firm with ingesta, and approximately 3 L of firm, dry, "stemmy" feces was removed through six enterotomies. Some of the fecal matter was very firm with superficial dark blood. The animal was recovered in her pen to encourage a return to normal behavior and diet, and pain was managed similarly. The warthog began eating small amounts of mash on day 15. On day 19 the animal had not had a bowel movement since surgery the week before, and 10 mg bisocodyl and cod liver oil were added s.i.d. On day 23 the animal was again immobilized for enemas and mineral oil gavage. Finally, on day 28 the animal was eating well and had a normal bowel movement. The diet was modified to discontinue the alfalfa, reduce the timothy hay offered to 2 handfuls b.i.d., and add 340 g of fruit and vegetables with 3 Tbsp. unprocessed bran (5 g fiber). No further constipation has been observed to date, and further diet modifications are under consideration.

Etiologies for constipation that are consistent with this case include: a reduced gastrointestinal (GI) transit time; dehydration; a GI toxin; an intestinal ileus; the administration of opioids; or dental disease. This warthog is an aging animal near the end of her life expectancy of 15 years. The incidence of constipation in humans increases with age and may be related to changes in the diet, most often due to decreased sensations of hunger and thirst that result in fewer calories, less water consumption, less fecal bulk, and a longer GI transit time.[1,2] Furthermore, anorexia and dehydration enhances colonic water absorption and leaves a dry, hard feces.[3] Alternatively, an intestinal ileus could have been initiated by a GI irritant, such as a saponin, or by an increased sympathetic tone from GI pain[4] or other stressors, such as the animal's osteoarthritic pain. The opioid that was used after both surgeries, although a modest dose, may have complicated the recovery of the bowel to normal function, however, the animal also appeared painful and was not drinking, which was more problematic. Dental disease needs to be considered due to the impaction of hay in the colon, however, this animal's teeth had been filed the previous year and there was no evidence of problems with mastication.

The subsequent diet modifications include the elimination of "stemmy" hay and the addition of fruit and a fiber supplement. Constipation commonly occurs in preparturient and lactating sows and is managed using feed ingredients with a high fiber content, such as beet pulp, oats, and wheat bran, as well as chemical laxatives, such as magnesium, potassium, or sodium sulfate.[5] Dietary fiber increases fecal bulk by providing indigestible matter and promoting fecal water holding and bacterial proliferation.[2] Fiber also increases stool weight, lowers colonic intraluminal pressure, and decreases whole gut transit time. Other useful bulking agents include psyllium, methylcellulose, and calcium polycarbophil. Low digestible carbohydrates with a low molecular weight, such as lactulose, also increase intestinal transit in constipation.[6] Severely constipated human patients often respond to polyethelene glycol solution, which may be more effective than pumpkin in future emergencies.[2] It is unclear whether the stimulant laxative, bisacodyl, was helpful in this case, postoperatively.

Three years earlier, another warthog at the North Carolina Zoo, an 11-year-old intact male, also suffered from periodic constipation that was successfully managed with mineral oil, pumpkin, and bran flakes. In light of these cases, geriatric captive swine may benefit from diet modifications that aid in preventing constipation.

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PASSERINE RICKETS IN A ZOOLOGICAL AVIARY

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Rickets is a metabolically induced bone disease found in some growing animals. Rickets can be caused by a variety of nutritional imbalances. Possible etiologies include inadequate intake of calcium, phosphorous, or vitamin D_3; or by inappropriate calcium to phosphorous ratios. Rickets caused by vitamin D_3 or calcium deficiency can be differentiated on histology. Recently, the North Carolina Zoological Park has incurred morbidity and mortality attributed to rickets in chicks of several species of passerine birds including red-faced liocichlas (*Liocichla phoenicea*), red-billed leiothrix (*Leiothrix lutea*), and snowy-headed robin chats (*Cossypha niveicapilla*). On histopathology, the osseous lesions were attributable to vitamin D_3 deficiency in some cases, while in others, the bony changes were considered to be secondary to hypocalcemia.

Rickets is a well described problem in wide variety of avian species that are maintained in zoological collections. It is primarily observed in young growing birds and altricial species are most prone to developing calcium deficiency since they undergo rapid growth. Some species of birds are capable of meeting the calcium demands of their chicks by selecting food items rich in calcium. Prevention is accomplished by providing an appropriate balance of nutrients. However, in captive settings this can be challenging due to a deficit in knowledge of the specific nutrient requirements for many avian species. Preparing well-balanced diets can be a complex task. Furthermore, an appropriately varied diet may be offered, but the birds may selectively feed their offspring limited items. Hand-rearing chicks to ensure adequate intake of nutrients can be problematic in captive breeding programs as it can lead to imprinting and subsequent loss of behaviors necessary for successful reproduction. Assisted feeding of the chicks by staff may also lead to nest disruption and abandonment by the parents.

The North Carolina Zoological Park was providing a standard diet for the affected avian species and husbandry conditions remained static. This talk was designed with the emphasis on discussing rickets in neonatal passerines, providing an overview of cases at the North Carolina Zoological Park, and for brainstorming on preventative measures appropriate for use in captive breeding programs where minimal nest disturbance is required.

CONUNDRUM V

Large mixed species exhibits provide some of the most complex nutritional challenges. Clinical and sub clinical diseases suggestive of mineral and/or vitamin imbalances, can manifest themselves in only one or a few species in such an exhibit. The clinical diseases may be observed only sporadically, and it is not uncommon for the husbandry teams of such exhibits to rule out nutritional influences as causes on the basis of there not having been any changes in diet for many years.

What are likely factors that could contribute to such a scenario?

How would you go about validating the contributions of these factors?

How can one prevent or minimize these factors to ward off clinical nutritional disease?

TRACKING DIETS IN ANIMALS: A STABLE ISOTOPE APPROACH

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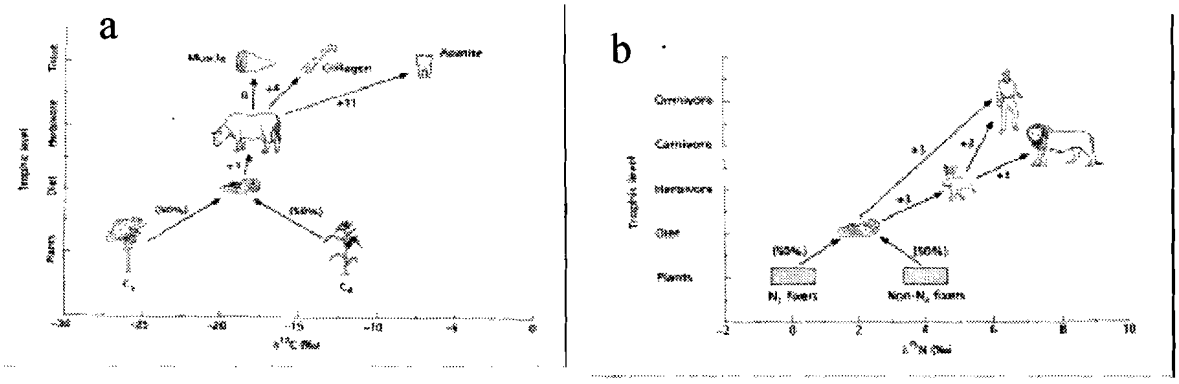
Stable isotopes are defined as "*any of two or more species of atoms of a chemical element with the same atomic number but with differing atomic mass or mass*" (Source: *Merriam-Webster's Medical Dictionary, © 2002 Merriam-Webster, Inc.*).

Stable isotopes of carbon (C), nitrogen (N), sulfur (S), oxygen (O) and hydrogen (H) occur naturally throughout the atmosphere, hydrosphere, lithosphere, and biosphere. In nature, 98-99% of these atoms are "light" isotopes (e.g. ^{12}C, ^{14}N and ^{16}O) while 1-2% are considered "heavy" isotopes (e.g. ^{13}C, ^{15}N and ^{18}O). These mass differences result in isotopic fractionation of the elements between phases during chemical and physical reactions. Isotopic measurement of these phases can yield information about the source of the element, the rates of the reaction, and the environmental conditions prevailing at the time of formation.

The Department of Soil Science at North Carolina State University houses a stable isotope mass spectrometry facility capable of measuring the stable isotopic composition of plant and animal tissue. Physical, chemical and biological reactions during processes such as photosynthesis, N-fixation, assimilation and metabolic activity can cause isotopic fractionation of the heavy and light isotopes in both plant and animal tissues. Animals derive their nutrition from plant and other animal sources. In general, the carbon isotopic composition of the food is conserved in the animal, while the nitrogen isotopic composition changes by ~+3‰ for every step in the trophic ladder. There is great interest in using the stable isotope ratios of carbon and nitrogen to (1) trace the sources (and relative contributions) of nutrients and (2) determine trophic level position of organisms in an ecosystem (Figure 1a and b, (Koch et al., 1994)).



These basic observations have helped researchers tackle conundrums of all sizes – from determining the progeny of different fish species in aquatic estuarine systems to the global migration patterns of birds. Recently, the stable isotopes of nitrogen have been used to study urea recycling in sheep (Sarraseca et al., 1998), man (Jackson, 1995) and other large mammals.

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OMEGA 3 FATTY ACIDS AND HIGH LEVELS OF VITAMIN A IMPROVE THE GROWTH (PART 1*.), AND THE HEALTH (PART 2#.) OF THE ENDANGERED WYOMING TOAD (*BUFO BAXTERI*)

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Introduction

Thirty two percent (32%) of the world's amphibians are threatened with extinction. In North America 55 species (21%) are threatened. The development of new methods in captive breeding programs is essential for these threatened species' long-term survival. The endangered Wyoming toad (*Bufo baxteri*) lives in southeastern Wyoming, a region characterized by short cool summers and by long cold winters. In captivity the Wyoming toad is often subject to poor growth, squamous metaplasia, as well as vitamin A deficiency in the liver. These problems are rare or absent in wild *B. baxteri* and can therefore be attributed to husbandry conditions including diet. Squamous metaplasia is the transformation of glandular or mucosal epithelium into stratified squamous epithelium, which compromises the adhesive properties of the tongue. Squamous metaplasia is a major cause of feeding problems and is associated with mortality of Wyoming toads. Squamous metaplasia has been associated with vitamin A deficiency, and *B. baxteri* livers often assay only 5% of the vitamin A found in wild toads. It is characteristic that thermo-conformers, including insects, living in cold regions have elevated levels of Omega 3 fatty acids. Omega 3 fatty acids are also associated with the transport and metabolic interactions of Vitamin A We tested the effect of a diet high in Omega 3 fatty acids and vitamin A on the growth and health of the Wyoming toad.

Experiment

This experiment tested three diets of crickets with different supplements on the growth and feeding ability of the Wyoming toads. There were four replicate tanks for each diet with three toads in each tank. The experiment began with early juvenile toads and lasted 24 weeks until the toads growth had slowed due to seasonal maturity. The toads' length and weights were measured every two weeks. Crickets were fed to satiation three times a week, and the number of crickets eaten recorded. Each diet was sampled at each feeding and the pooled amount from each diet individually analyzed for proximate components, Vitamin A, and fatty acid profiles. At the end of the experiment the feeding ability of the toads was measured by; 1) counting the number of strikes at crickets, and 2) counting the number of crickets ingested by each toad over a five-minute interval. The rate of the removal of the vitamin and Ca/D powder by crickets was measured.

Enrichment Diet

The Enrichment Diet (high in Omega 3 fatty acids and vitamin A) consisted of 8 mm crickets that were fed an enrichment mixture and then dusted with powered calcium and vitamin D powder (Ca/D). The enrichment mixture combined high protein ground aquatic turtle food (Mazuri®, Brentwood MO, USA), spirulina (Spirulina Powder® Spirulina Powder, Now Foods,

Bloomingdale, IL, USA), and fish oil (Omega Maintenance® Salmon Oil, Arctic Paws, LLC, Anchorage, AK, USA); by weight 77% turtle feed , 11% spirulina powder, and 12% salmon oil. Crickets were fed the enrichment mixture for thirty minutes, then were dusted with Ca/D powder, and then fed to the toads within five minutes. **Vitamin Diet**: The Vitamin Diet consisted of crickets given a dusting with a complete vitamin and mineral powdered mixture (Reptivite® Zoo Med, San Luis Obispo, CA,) one day weekly. On the other two days the crickets were dusted with Ca/D. **Ca/D Diet**: The Ca/D Diet was crickets dusted only with Ca/D (Herpcare® Virbac AH Inc., Fort Worth, Texas, USA) and fed on all three feed days (Table 1).

Table 1. Weekly schedule of cricket treatments.

	Monday	**Wednesday**	**Friday**
Enrichment Diet	Enrichment[1] + Ca/D[2]	Enrichment[1] + Ca/D[2]	Enrichment[1] + Ca/ D[2]
Vitamin Diet	Vitamin powder[2]	Ca/D[2]	Ca/D[2]
Ca/D Diet	Calcium/ Vitamin D (Ca/D)[2]	Ca/D[2]	Ca/D[2]

[1]Supplement was fed to crickets 0.5-1.0 hr prior to feeding crickets to toads
[2]Supplement was dusted onto crickets within 5 minutes of feeding crickets to toads

The analysis of the diets showed that the Enrichment Diet was similar to the other diets for most nutrients, except that the Ca/D Diet had a higher 2.5:1.0 Ca/P ratio than the others (2.0/1.0), and that the Enrichment Diet was 400% higher in Vitamin A and 200% higher in Omega 3 fatty acids than the other diets (Table 2).

Table 2. The percentages of Omega 3 fatty acids in the three diets.

Acid Profile	**Enrichment Diet**	**Vitamin Diet**	**Ca/ D Diet**
Docosahexaenoic (DHA) C22:6n3c	1.80%	0.20%	0.00%
Docosapentaenoic C22:5n3c	0.46%	0.00%	0.00%
Eicosapentaenoic (EPA) C20:5n3c	2.81%	1.10%	0.96%
Omega 3 Fatty Acids	5.89%	2.85%	2.86%

Toads fed the Enrichment Diet, grew faster in weight ($P<0.01$) than those on the alternate diets. The Vitamin Diet had a significantly lower growth rate than the other diets ($P<0.03$, Table 3).

Table 3. The change from initial to final weight and length (FWt–IWt; FL–IL) respectively, the weight of crickets eaten (MC), the food conversion ratio (FCR), and the specific growth rate in weight (SGR^{mass}) and snout-urostyle length (SGR^{SUL}) over the 24 week period.

Treatment	**FWt-IWt (g)**	**FL-IL (mm)**	**MC (g)**	**FCR**	**SGR^{weigth}**	**SGR^{SUL}**
Enrichment Diet	26.5 ± 0.7b	16.2 ± 0.86	121.5 ± 9.1	4.97 ± 0.90	0.86 ± 0.02%c	0.21 ± 0.01%
Vitamin Diet	20.8 ± 0.3a	14.4 ± 0.84	105.5 ± 0.7	5.41± 0.43	0.74 ± 0.04%a	0.19 ± 0.01%
Ca/D Diet	25.7 ± 1.7b	15.0 ± 0.36	121.1 ± 8.2	4.65± 0.33	0.80 ± 0.02%b	0.20 ± 0.06%
Probability	P< 0.01	P<0.26	P<0.24	P<0.34	P<0.03	P<0.40

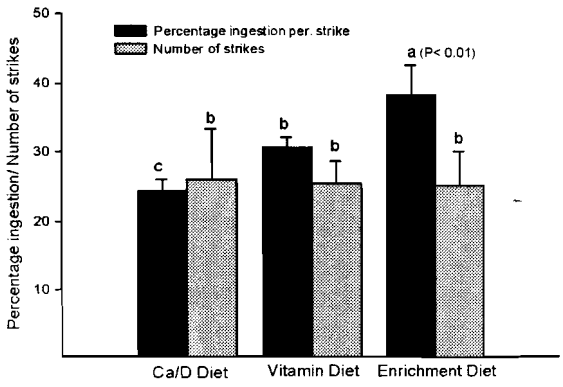

Figure 1. Comparison of strike and ingestion rates between the three diets

Approximately 50% of both the Ca/D and vitamin powders were removed from the crickets within five minutes of dusting.

Conclusion

The Enrichment Diet provided an improved growth rate over the Vitamin Diet, and improved feeding over the alternate diets. This may be related to a higher consumption of Omega fatty acids and Vitamin A provided by the Enrichment Diet. A further improved diet for anurans could include the addition of vitamin mixtures to the enrichment mixture and further refinement of the mixture's composition.

2ND ANNUAL CRISSEY ZOOLOGICAL SYMPOSIUM UPDATE

We were very excited about the continued success of the Crissey symposium during its 2nd year. Dr. Ed Stevens was an engaging keynote speaker and we had numerous very interesting presentations regarding carnivore nutrition that encouraged needed dialogue amongst attendees. Our general nutrition section was also very well received.

In addition, we were excited to start a new tradition within the symposium: The Conundrum Discussions. We were pleasantly surprised by the discussions generated by the conundrums and we hope that you were as well. We have summarized these discussions in the following document both for your review and for your input. Please contact Mrs. Ange with any feedback that you have regarding these topics.

Last year we had several people give us needed critiques regarding the conference such as providing a finalized schedule of events much earlier than we have previously as well as trying to rotate the conference among several key institutions that were special to Dr. Crissey. We greatly appreciate this feedback and hope to be able to incorporate these ideas in the upcoming years as we add more people to the committee organization board. We genuinely welcome your feedback as well in order to help us make this symposium an even larger success.

Sincerely,

Kimberly Ange-van Heugten & Michael Stoskopf

CONUNDRUM 1:

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

Discussion: Questions were posed as to how good is the clinical information currently available showing that protein is bad for the kidney. There was some suggestion for the group to read a dog research study by Campbell on this topic.

It was suggested that a study with different clinical diets and a large number of animals is needed. Both BUN and creatinine increase with hi protein diets but this is only significant in many people's mind if these values increase higher than they were previously reported. The kidney could be compromised before the diet change and therefore BUN and creatinine values may not help the researcher and the question was posed: Do we really know what stresses the kidney for all animals (Ca, P, N)?

More questions were asked in theory: Is it best to feed squid (it is lower in P)? Is it best to feed penguins fish that are low in protein? Does the state of the kidney disease matter (acute versus chronic conditions)? Are many of these sick animals forced to make their own protein via catabolism and therefore further compromised? Is there a total amino acid approach that can be used? Is switching their food at a compromised state making a bad situation even worse? Are institutions using this no protein concept for omnivores, too and if so can we stop it?

This conundrum evoked a lot of discussion and most of it was generated to ask future questions.

CONUNDRUM II

Topic Posed: What is a good forage?

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

Discussion: There was immediate discussion as to the fact that alfalfa is not responsible for the condition referred to as "tying up" and that perhaps similar conditions to "tying up" were being misevaluated. In addition it was suggested that many of the conditions associated with alfalfa were not proven to be linked to feeding it and that this should be proven instead of just possibilities.

There was also discussion that many people were feeding lower quality hay because they were scared of some of the rumored alfalfa problems and that this was likely to cause more problems than alfalfa ever would. In addition it was mentioned that hay quality is incredibly variable especially amongst the numerous institutions represented by the conference population.

It was generally agreed that overfeeding alfalfa was a problem.

To evaluate the quality of forages it was suggested that it was best to look at the fields before the product was harvested (although this is obviously not reasonable for all institutions represented).

It was pointed out that non-domestic equids would not eat lush feeds such as alfalfa in the wild. They would eat grass hay that is approximately 7% CP and poor quality.

The question was posed: What is quality? We measure CP, fiber, etc. but do any of these one measures make the forage = high quality. We should look at the measurements in multiple dimensions and not on a linear basis. Perhaps better than the measurement of what is in the feed is the measurements of how each species perform on a particular feed is more important?

It was pointed out to remind the audience that there is a huge difference between Bad Hay & Poor Quality hay. Bad = contaminated.

Hay was also discussed in association with the enterolith issues facing numerous species. It was reinforced that many factors associated with forages can present issues (both good and bad): tannins, gut fill, enrichment, nutritional needs, etc.

CONUNDRUM III

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

Discussion: The first idea posed was whether there is any way that research or select housing at some institutions could be conducted using "real" seawater. Most people attending the conference did not feel this was possible although agreed it would be helpful. The second research idea was to use the same diet in as many enclosures as possible to try and void diet as a variable to ascertain other problem aspects.

It was suggested that institutions should further research the differences between regular salt (NaCl) and the salt mixes. Most people agreed that there seem to be large differences between the different sea salts marketed.

The question/theory was posed that the problems seen may have nothing to do with diet and that it is all disease.

It was also suggested to do more frequent x-rays and preventative care techniques for animal species that are known to be especially susceptible to this problem.

CONUNDRUM IV

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

Discussion: This topic generated a large response of both answers and potential ideas. It was suggested that visual body condition scores be developed for all species and to have these documented and then circulated amongst zoos. The small wonders of training were also discussed and how often animals can be trained to do things we never thought possible. This discussion particularly involved how marmosets and birds have been trained to weigh themselves easily.

The unique problem of shelled animals was also discussed. How BCS cannot be used with animals such as lobsters and turtles.

It was mentioned that weight is not always a good indicator because an animal of average weight could still have poor musculature, etc. In addition, some animals can be very sick without losing any weight and that when they finally loose the weight it may be too late anyway.

It was also mentioned that perhaps a measure of weight is not as valuable as many people think. Perhaps getting information from urine and feces can be a better (and easier to acquire) assessment tool. Valuable information is also available via hair samples and these samples can even provide long-term nutritional status information.

Several people agreed that sometimes we get too caught up with weight and that if the animals are behaving normally and reproducing normally that they are healthy despite a potential weight concern.

CONUNDRUM V

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

Discussion: This question evoked an enormous amount of discussion although the evaluation question was not really addressed.

In general it was agreed that the waste candy blocks provided a very unhealthy environment for bears due to 1) the obvious presence of the hunters & 2) the potential bacterial cultures, dental carries, and other health issues. Diarrhea and diabetes concerns were also mentioned. Some of the most suggested worries regarding these candy blocks also included: behavior changes (bear aggressiveness and possessiveness), disease spread, and home range changes that take the animals closer to people's homes. Is this a potential threat to humans (or is the waste block itself a potential disease housing area that could threat humans). This problem has been notice in the wild with female bears seeming to get pregnant earlier than normal when living closer to these blocks.

The problem with these candy blocks altering the entire environment was also discussed. The change to the bug population within areas was thought to be a huge but underestimated problem.

The final thought was that this situation is (or at least should be) considered an embarrassment for the people trying to bait the animals. It reduces the quality of life for all animals involved and should not be a maintained event.

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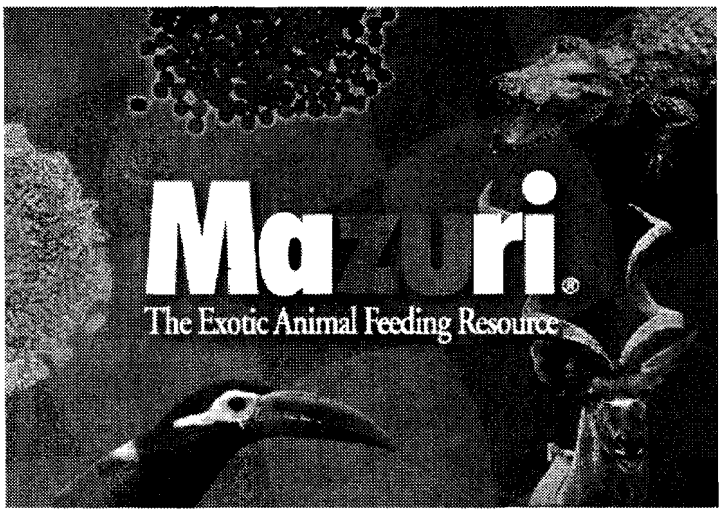

http://mazuri.com/

AQUATIC DIETS

Mazuri® Auklet Vitamin 52M5

Mazuri® Aquatic Gel Diet 5M70

Mazuri® Aquatic Gel Diet for Crustaceans 58RR

Mazuri® Low Fat Aquatic Gel Diet 5ME2

Mazuri® Fresh Water Turtle Diet - 0.5 lb 5E08

Mazuri® Fresh Water Turtle Diet 5M87

Mazuri® Herbivore Aquatic Gel Diet 5Z93

Mazuri® Koi Platinum Bits 5M81

Mazuri® Koi Platinum Bits - 1.5 lb. 5E04

Mazuri® Koi Platinum Nuggets 5M80

Mazuri® Koi Platinum Nuggets - 4.25 lb. 5E03

Mazuri® Koi Platinum Ogata 5MC8

Mazuri® Koi Platinum Wheat Nuggets 50D0

Mazuri® Koi Pond Nuggets 5M78

Mazuri® Omnivore Aquatic Gel Diet 5Z94

Vita-Zu Sharks/Rays Vitamin Supplement Tablet 5M24

Vita-Zu Sharks/Rays II 5MD8

MARINE MAMMAL DIETS

Vita-Zu Mammal Tablet Vitamin Supplement 5M26

Vita-Zu Mammal Tablet w/out Vitamin A 5TLA

WATERFOWL/PHEASANT DIETS

Mazuri® Exotic Gamebird Starter 5637

Mazuri® Exotic Gamebird Maintenance 5643

Mazuri® Exotic Gamebird Breeder 5639

Mazuri® Sinking Waterfowl Maintenance 5MI3

Mazuri® Waterfowl Breeder 5640

Mazuri® Waterfowl Starter 5641

Mazuri® Waterfowl Maintenance 5642

Mazuri® Sea Duck Diet 5681

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