Zoo and Wildlife Nutrition Foundation

ZWNF

and

Nutrition Advisory Group

ASSOCIATION OF ZOOS & AQUARIUMS NAG

Eleventh Conference on Zoo and Wildlife Nutrition

27-30 September 2015
Portland, Oregon, USA

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Proceedings of the

Eleventh Conference

of the

Zoo and Wildlife Nutrition Foundation (ZWNF)

and

Association of Zoos and Aquariums (AZA)

Nutrition Advisory Group (NAG)

on

Zoo and Wildlife Nutrition

Edited by
Heidi Bissell and Matt Brooks

27-30 September 2015
Portland, Oregon, USA

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The Conference Committee wishes to express our sincere thanks to the many people who were instrumental forces behind the eleventh conference on zoo and wildlife nutrition. Again, we would like to thank AAZV for the opportunity to meet simultaneously with them in order to encourage camaraderie and information sharing between the groups.

2015 AZA Nutrition Advisory Group Steering Committee

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Cover artwork was generously provided by the Metro Toronto Zoo.

Previous Conference Locations:

1995	Scarborough, ON Canada
1997	Fort Worth, TX
1999	Columbus, OH
2001	Lake Buena Vista, FL
2003	Minneapolis, MN
2005	Omaha, NE
2007	Knoxville, TN
2009	Tulsa, OK
2011	Kansas City, MO
2013	Salt Lake City, UT
2015	Portland, OR

September 2015

Dear Friends and Colleagues,

It is with great pleasure that we welcome you to the Zoo and Wildlife Nutrition Foundation and AZA Nutrition Advisory Group's Eleventh Conference on Zoo and Wildlife Nutrition in conjunction with the American Association of Zoo Veterinarians (AAZV). The field of zoo nutrition continues to advance and the ongoing importance of science-based nutrition decision making remains paramount.

Again for our eleventh conference, we meet in conjunction with AAZV. Meeting together, we continue to work towards a common goal of good health for the animals in our care via enhanced communication and collaboration with a focus on the science of nutrition. We would like to especially thank Rob Hilsenroth, Adine Nicholson, and the rest of the AAZV team for their assistance and logistical support as we developed the meeting plan.

For this year, we continue the exciting additions from last conference, designed to enhance involvement and participation. Case studies will continue the increased emphasis on thought process development, discussion, and information sharing among the session attendees. With such a small group of participants at the meeting, we hope to foster an atmosphere of sharing and meaningful interactions – both in the meeting sessions and out.

We continue to encourage all of you to get involved in the NAG by becoming an advisor to one of the SSP/TAG programs, working together with others in advising, writing or reviewing one of the animal care manual nutrition sections, working to help revise/review the website, and/or working to make the next conference bigger and better. Additionally, there is a need for everyone to participate in critical, scientific review of nutrition/health topics currently in the scientific and popular press. As nutrition experts, we need to work collaboratively to address these issues and dispel any myths and misinformation with the application of valid science.

Our conference is the result of hard work completed by several dedicated people. We want to thank Matt Brooks for chairing the conference, Heidi Bissell for defining the program, Brian Rude for overseeing the Roy McClements Student/Keeper Competition, Jennifer Parsons for spear-heading the workshop, Kerri Slifka for coordinating the incredible fund-raising efforts, Laura Franske for keeping us social, and everyone involved in reviewing, editing, and assembling the proceedings. The conference committee is listed herein, and the efforts of all of these folks are much appreciated.

We received significant financial support from Rodent Pro, Central Nebraska Packing, Mazuri, and Zukudla, among many others. Please make a special effort to thank their representatives and all of those who supported the conference while you're here. Also be sure to thank the exhibitors by visiting them, collecting information, assessing it, and making purchases, as appropriate. They support us with new information and technology that we can use to do our jobs well.

Finally, we want to thank you for attending this meeting. In this time of tight finances and time schedules, please take advantage of the time you have invested in traveling here to share your experience and knowledge, gain the same from others, and continue to build a better advisory group. Thank you!

Mike Maslanka, M.S., Chair, AZA Nutrition Advisory Group
Barbara Henry, M.S., President, Zoo and Wildlife Nutrition Foundation

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Thank you!


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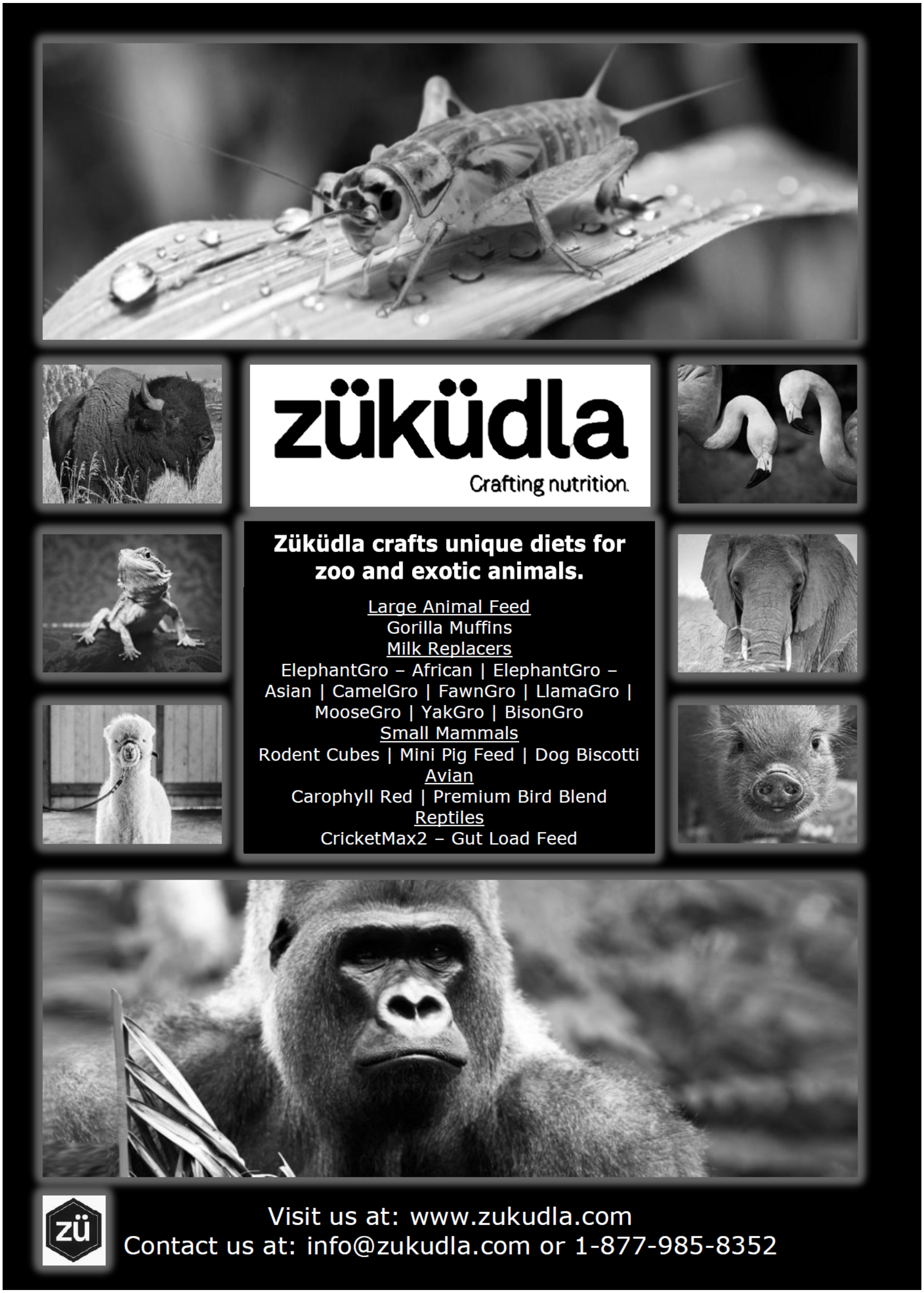
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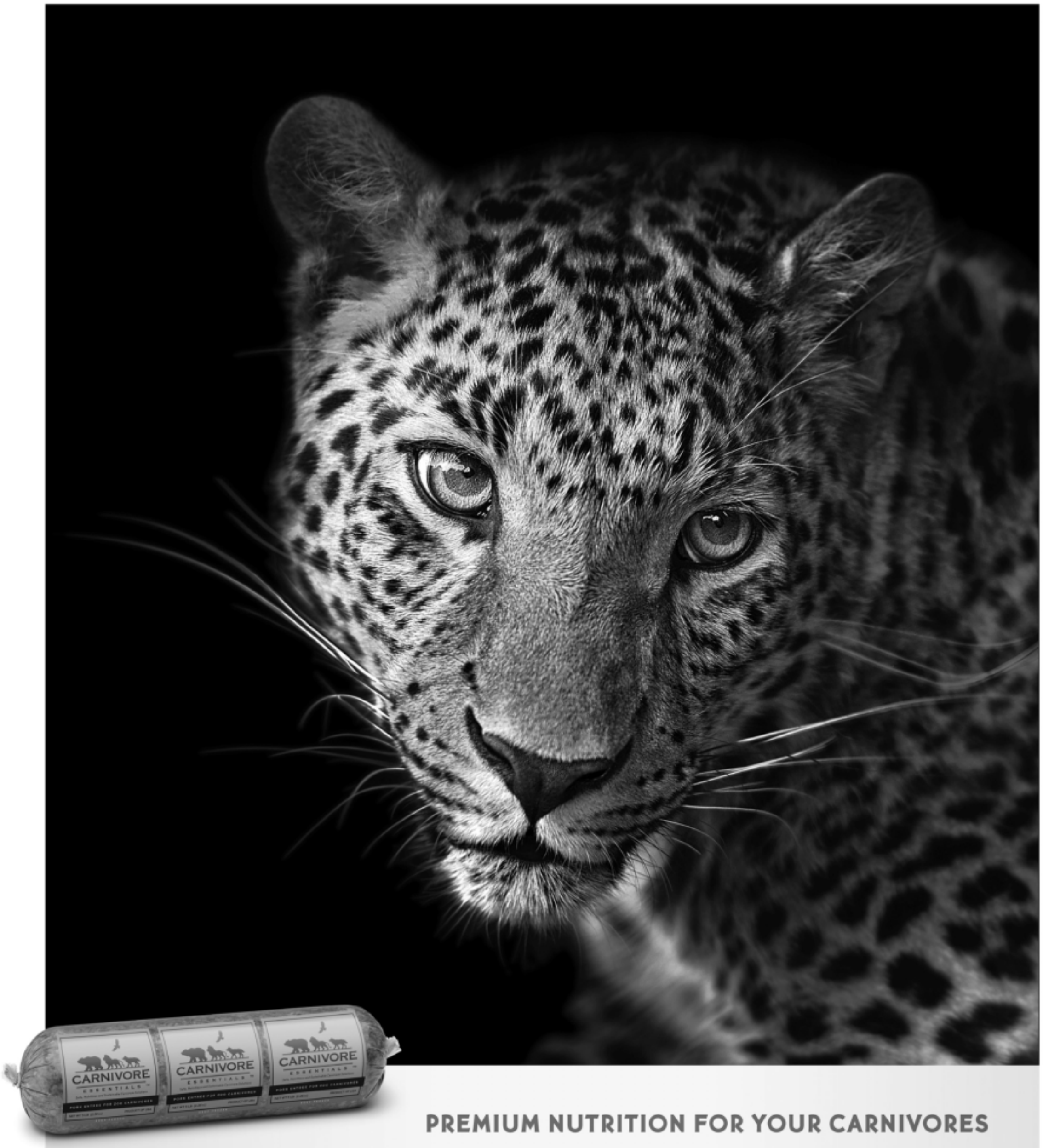
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The primary role of SCARF is to support a residency program in zoo and wildlife nutrition. This multi-year program allows a post-graduate student to get hands-on nutrition experience. For the ZWNF and the NAG, this program represents one of the concrete ways that we are training the next generation of zoo nutrition leaders.

Donations to the program are welcome at any time. See a steering committee member or the NAG website (www.nagonline.net) for details.

Details of the SCARF benefit event, sponsored by Central Nebraska Packing, Inc. and Zükúdla, will be posted at the time of registration.

Nutrition Advisory Group
Eleventh Conference Schedule
26 – 30 September 2015
Red Lion Hotel on the River, Portland, Oregon

Saturday, 26 September 2015

4:30 - 5:30 PM	Registration	Lobby
6:00 PM	Steering Committee Meeting	

Sunday, 27 September 2015

8:00-9:00 AM	Registration	Lobby
9 AM - 4:30 PM	**Workshop: It's not about the blueberries: behavior-centered food enrichment** *INVITED SPEAKER: Jessica Sheftel* *Presenters: Matt Brooks, Katie Kerr, Jennifer Parsons*	Oregon Zoo *Bus departs hotel at 9 am* *Returns by 4:30 pm*
6:30 PM	**Icebreaker with AAZV**	River Deck and Patio

Monday, 28 September 2015

7:00 - 8:00 AM	Continental Breakfast	Lobby
7:30 - 8:00 AM	Registration	Lobby
8:00 - 8:20 AM	Introductions, welcome, opening with AAZV	East Salon/Grand Ballroom
	Primate Gut Health and Nutrition Part I *Session Chairs: Barbara Henry and Adam Reppart*	Timberline
8:20 AM	**Fruit-free diets for primates** *INVITED SPEAKER: Amy Plowman*	
9:00 AM	**Stronger than yesterday: New nutrient recommendations for lorisine primates** *Francis Cabana*, Ellen Dierenfeld, Giuseppe Donatti, and K.A.I. Nekaris* **Participant in the Roy McClements Student-Keeper Paper Competition#**	
9:20 AM	**Assessment of apple leaves and bark as suitable browse items for herbivorous monogastric species under human care** *Sarra Gourlie*, Jim Atkinson, and Jaap Wensvoort*	
9:40 AM	**Case Study: Unintended consequences of addressing GI distress in a siamang (*Symphalangus syndactylus*)** *Erin Kendrick, MS* and Mike Maslanka, MS*	

10:00 -10:30 AM	**BREAK and POSTER SESSION**	**AAZV:** East Salon Hallway of the Grand Ballroom **NAG**: Mt. St. Helens Ballroom Lobby

	Primate Gut Health and Nutrition Part II *Session Chairs: Barbara Henry and Adam Reppart*	Timberline
10:30 AM	**Medical nutrition therapy for human gastrointestinal disorders and application to captive non-human primates** *INVITED SPEAKER: Adam Reppert, MS, RD*	
11:10 AM	**Associations between diet, gut microbial communities, and health in red-shanked doucs (*Pygathrix nemaeus*): A model for the subfamily colobinae** *J. B. Clayton, D. Knights, F. Cabana*, H. Huang, L. T. Ha, T. V. Bui, M. V. Vo, D. A. Travis, and T. J. Johnson*	
11:30 AM	**Nutritionally complete food-free diets for primates: Potential benefits and concerns** *Barbara A. Henry, MS* and Adam Reppert, MS, RD*	
12:00 PM	**LUNCH ON YOUR OWN**	

1:30 - 3:10 PM	***<u>Nutrition Career Panel, Part I</u>*** *Session Chair: Barbara Toddes* *Panelists: Michael Power, Michael Schlegel, Shannon Livingston, Clif Martel, and Mark Edwards*	Timberline
3:10 - 3:40 PM	**BREAK and POSTER SESSION**	**AAZV**: East Salon Hallway of the Grand Ballroom **NAG**: Mt. St. Helens Ballroom Lobby

3:40 - 5:30 PM	***<u>Nutrition Career Panel, Part II</u>*** *Session Chair: Barbara Toddes* *Panelists: Michael Power, Michael Schlegel, Shannon Livingston, Clif Martel, and Mark Edwards*	Timberline
5:00 PM	**Student debt relief** *INVITED SPEAKER: Amelia Lockhart*	
5:30 PM	**DINNER ON YOUR OWN**	
7:30 - 9:00 PM	**SCARF BENEFIT** with <u>live-action</u> "Hungry Hungry Hippos"	JB Lounge

Tuesday, 29 September 2015

7:00 - 7:45 AM	Continental Breakfast	Lobby

	<u>Herbivores</u> *Session Chair: Katie Sullivan*	Timberline
7:45 AM	**An analysis of vitamin C supplementation in the drinking water for giant elephant shrews (*Rhynchocyon petersi*) at the Philadelphia Zoo** *Amanda Christine Steinagel*, Barbara Toddes, Donna M. Ialeggio, and Benjamin Golas* **Participant in the Roy McClements Student-Keeper Paper Competition**[#]	

8:05 AM **Evaluation of diets offered to elephants in Brazilian zoos**
*Lucas Carneiro, Ana Raquel Faria , Gabriel Werneck, and Ellen S. Dierenfeld**

8:25 AM **Use of a novel iron chelator (HBED) in black rhinoceros**
Kathleen E. Sullivan, Eduardo V. Valdes, Shannon E. Livingston, Mitchell D. Knutson, Charles R. Staples, Lori K. Warren, and Shana R. Lavin*
Participant in the Roy McClements Student-Keeper Paper Competition[#]

8:45 AM **Vitamin E supplementation in African elephants**
Katherine Kerr, Kathleen Sullivan, Shannon Livingston, Charles Staples, and Eduardo V. Valdes*

9:10 AM **Case Study: A hemolytic event in an iron overloaded black rhinoceros (*Diceros bicornis*) in association with cessation of chelation therapy**
Kathleen E. Sullivan, Natalie D. Mylniczenko, Jessica A. Emerson, Natalie H. Hall, Deidre Fontenot, Ryan De Voe, Elizabeth Nolan, Nicole Stacy, Shannon E. Livingston, Shana R. Lavin, Eduardo V. Valdes, and Geoffrey W. Pye*

9:35 - 10:05 AM **BREAK and POSTER SESSION** **AAZV**: East Salon Hallway of the Grand Ballroom
NAG: Mt. St. Helens Ballroom Lobby

Nutrition Ops Timberline
Session Chairs: Matt Brooks and Devan Compart

10:05 AM **Creation of the zoo nutrition and wildlife foundation**
Barbara A. Henry, Michael T. Maslanka, Ann M. Ward, Shannon Livingston, Howard Fredrick, Kerri A. Slifka, and Barbara Toddes*

10:25 AM **Financial aspects of zoo diet management**
Mike Maslanka

10:45 AM **Food safety training for keepers in decentralized zoo kitchens**
Devan Paulus Compart

11:05 AM **The tools we use: Outfitting your operation for efficacy and efficiency**
Matthew A. Brooks, Ph.D. and Michael T. Maslanka, MS*

11:25 AM **Tools and techniques for successfully grinding fish for tube feed formulas**
Juliet Eckert

12:00 PM **ZOO DAY** Oregon Zoo

Wednesday, September 30

7:00 - 7:45 AM Continental Breakfast Lobby

AAZV-NAG Joint Session: Herptile Nutrition East Salon/Grand Ballroom
Session Chairs: Eduardo Valdes and Eric Klaphake

8:00 AM **Introduction - Susie Bartlett**

8:02 AM **Evaluation of vitamin A status and diagnosis of hypovitaminosis A in amphibians**
Allan P. Pessier, DVM, Dipl ACVP and Carlos E. Rodriguez, DVM, Dipl ACVP*

8:18 AM **Understanding the interactions of diet and lighting on frogs and their symbiotic bacteria to improve ex situ husbandry of amphibians**
*Rachael E. Antwis, PhD, Christopher J. Michaels, PhD, Richard Preziosi, PhD, and Andrea L. Fidgett, PhD**

8:30 AM **Considerations to maximize nutrient supplementation of feeder insects**
Shannon E. Livingston, MSc, Kathleen E. Sullivan, MS, and Eduardo V. Valdes, PhD*

8:42 AM **Non-healing subcutaneous hemorrhage in a colony of vampire bats (*Desmodus rotundus*) due to suspected vitamin C deficiency**
Jennifer C. Hausmann, DVM, Jorden Manasse, BVMS, Howard Steinberg, VMD, PhD, Dipl ACVP, Victoria L. Clyde, DVM, and Roberta Wallace, DVM*

8:54 AM **Body weight changes of leopard tortoises (*Stigmochelys pardalis*) fed two isoenergetic diets**
Breanna P. Benson and Mark S. Edwards, PhD*

9:06 AM **Evaluation of the nutritional status of rehabilitated green sea turtles (*Chelonia mydas*) utlizing nutritional markers, stable isotopes, and metagenomics**
Jennifer C.G. Bloodgood, MS, Terry M. Norton, DVM, Dipl ACZM, Lisa A. Hoopes, PhD, and Sonia M. Hernandez, DVM, Dipl ACZM, PhD*

9:18 AM **Ex situ amphibian nutrition: recent findings and future directions**
Elizabeth A. Koutsos, MS, PhD and Eduardo Valdes, MS, PhD*

9:34 AM **Questions and Answers**

9:45 - 10:30 AM **BREAK** Mt. St. Helens Ballroom

Carnivores, Part I Timberline
Session Chair: Katie Kerr

10:30 AM **Cheetah nutrition: Recent advances and revised SSP recommendations**
Ellen Dierenfeld, Katherine Kerr, and Katherine Whitehouse-Tedd*

10:50 AM **Utilization of pork and pork by-products for nutritional management of captive exotic felids**
Cayla Iske, Cheryl Morris, and Kelly Kappen*

11:10 AM **Feeding strategies in wild carnivores: Progress report of a model approach**
Annelies De Cuyper, Marcus Clauss , Myriam Hesta, and Geert P.J. Janssens*
Participant in the Roy McClements Student-Keeper Paper Competition#

11:30 AM **A comparative nutrient analysis of fish species consumed by managed and free-ranging common bottlenose dolphins, *Tursiops truncatus*, with respect to ammonium urate nephrolithiasis**
Amanda J. Ardente, Richard C. Hill, Karen C. Scott, Brian J. Vagt, and Randall S. Wells*

11:50 AM **A targeted metabolomics assay to measure purines in the diet of managed and free-ranging common bottlenose dolphins, *Tursiops truncates***
Amanda J. Ardente, Timothy J. Garrett, Karen C. Scott, and Richard C. Hill*

12:10 - 1:15 PM **LUNCH ON YOUR OWN**

Carnivores, Part II — Timberline
Session Chair: Katie Kerr

1:15 PM — **Hypovitaminosis A: Influence of three diets or topical treatment on hepatic, adipose, and plasma retinoid concentrations and presence of squamous metaplasia in Mississippi gopher frogs (*Rana capito servosa*)**
Christina Ploog, Robin Clunston, Cheryl Morris, Cayla Iske, William Blanner, and Allen Pessier*

1:35 PM — **A review of the nutrient content of commercial feeder insects and strategies for increasing their nutrient content**
Mark Finke

1:55 PM — **Nutrient composition of the milk of the giant anteater (*Myrmecophaga tridactyla*)**
Michael Power

2:20 PM — **Case Study: Anorexic Anteater**
Heidi Bissell

2:30-3:15 PM — **BREAK** — Mt. St. Helens Ballroom

General Topics — Timberline
Session Chair: Heidi Bissell

3:15 PM — **A different angle on geometrical analysis of diets: Developing a tool for using geometry to communicate nutrition to laypeople**
Heidi Bissell

3:35 PM — **Case Study: What goes around, comes around. The case for transfaunation**
Mike Maslanka, MS and Amanda Guthrie, DVM, Dipl. ACZM*

4:00-5:00 PM — **MEMBERSHIP MEETING including program updates and conference summary**
Mike Maslanka, MS and Barbara Henry, MS

6:00 PM — **NAG BANQUET** — JB Lounge

[#]The Roy McClements Student-Keeper Paper Competition is sponsored by
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Visit with the authors Monday 9/28 at 10:00 am and 3:10 pm and Tuesday 9/29 at 9:35 am.

Analysis of nutrients, vitamin stability, and moisture loss in primate browse harvested at the San Diego Zoo
Rhianne M. Maxwell, BS and Michael L. Schlegel, PhD, PAS, Dipl ACAS*
Participant in the Roy McClements Student-Keeper Paper Competition#

Birth weights and growth rates of giraffe and okapi at Disney's Animal Kingdom
Shannon Livingston, Kathleen Sullivan, Katherine Kerr, and Eduardo Valdes*

Calibration development for rapid assessment of fish species for dolphins in human care using near infrared reflectance spectroscopy (NIRS)
Scott Williams, Kathleen Sullivan, Shannon Livingston, Katherine Kerr, and Eduardo V. Valdes*

Commercial aquatic diets and water quality
Katherine Kerr, Matthew Dawson, Kathleen Sullivan, Amber Thomas, Shannon Livingston, Andy Stamper, Charles Staples, and Eduardo V. Valdes*

Common aquatic ingredient nutrient analyses: balancing practical feeding with long term aquatic health
Kathleen Sullivan, Shannon Livingston, Katherine Kerr, Scott Williams, and Eduardo V. Valdes*

Designing an online hand-rearing resource center: first steps
Gail E. Hedberg, RVT, Kimberly Thompson, DVM, Laurie J. Gage, DVM, Louise Peat, Kimberly Robertson, and Ellen S. Dierenfeld, PhD*

Effect of dietary soluble fiber on gut microbiota in the sugar glider (*Petaurus breviceps*): a pilot study
Lindsey Solden, Ellen S. Dierenfeld, and Kelly Wrighton*

Evaluating the nutrition of a flock of non-breeding great flamingos (*Phoenicopterus ruber roseus*) at the San Diego Zoo Safari Park
Michael L. Schlegel, PhD, PAS, Dipl. ACAS-Nutrition, Angela Ray, BS, and Jennifer Tibbot, BS*

Evaluation of a commercial vitamin and mineral supplement in milk replacers on serum nutrients in pre-ruminants
Michele Gaffney, MS and Michael L. Schlegel, PhD, PAS, Dipl. ACAS*

Evaluation of browse composition: Vitamin E
Katherine Kerr, Kathleen Sullivan, Shannon Livingston, Charles Staples, and Eduardo V. Valdes*

Feeding Frenzy: Turning the science of zoo nutrition into a game for kids
Bethany Fisher and Heidi Bissell*
Participant in the Roy McClements Student-Keeper Paper Competition#

The growth responses of two polar bear (*Ursus maritimus*) cubs to regimented dietary energy
Jaap Wensvoort, Gregory Thiemann, Dawn Mihailovic, Jim Atkinson, and Ian Duncan*

Hand-rearing and growth of a grey seal (*Halichoerus grypus*)
Erin Kendrick, Michael Maslanka, and Rebecca Sturniolo*

Impact of dietary *n*-3 and *n*-6 PUFA on oxidative status and inflammation in yellow-rumped warblers
Morag F Dick, Laura A. Rooney, and Christopher G. Guglielmo*
Participant in the Roy McClements Student-Keeper Paper Competition#

Interpreting vitamins and mineral concentrations in serum of exotic species: Lab values are not infallible
Kathleen E. Sullivan, Shannon Livingston, Katherine Kerr, and Eduardo V. Valdes*

Investigation of a new diet formula for captive short-beaked echidnas (*Tachyglossus aculeatus*)
*Hayley J. Stannard, Jamie M. Bekkers, and Michelle Shaw**

Managing diabetes in black-footed tree rats (*Mesembriomys gouldii*) and greater stick-next rats (*Leporillus conditor*) through diet
*Corinne Lendon, Frances Hulst, and Michelle Shaw **

Nutritional analysis of mixed produce for exotic species using near infrared reflectance spectroscopy (NIRS)
Scott Williams, Kathleen Sullivan, Shannon Livingston, Katherine Kerr, and Eduardo V. Valdes*

Preliminary investigations into circulating vitamin E concentrations in captive okapi (*Okapia johnstoni*)
Kerri Slifka, MS and Janis Raines, DVM*

So you (don't) think you need a nutritionist
Mike Maslanka, MS, Barbara Henry, MS, and Ann Ward, MS*

#The Roy McClements Student-Keeper Paper Competition is sponsored by
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In order of first author's last name

FRUIT-FREE DIETS FOR PRIMATES

Amy Plowman

Whitley Wildlife Conservation Trust (Paignton Zoo Environmental Park), Totnes Road, Paignton, Devon TQ4 7EU, U.K.

Abstract

Paignton Zoo has been working towards fruit-free diets for primates since about 2003, when severe dental health issues in our colobus monkeys (*Colobus guereza*) triggered us to investigate sugar levels in their diet. In common with most of our primates at the time their diet consisted of a commercial primate pellet and included a high proportion of fresh and dried fruit, along with vegetables, bread and seeds. From our investigations it became apparent that orchard grown fruit, selected to be palatable to humans, contains significantly more non-structural carbohydrate (sugars) and less fibre than wild fruits consumed by free-living primates. The difference is even more extreme when comparing orchard fruits with the leaves typically consumed by folivorous primates. So we took the decision to remove fruit completely from the diets of the colobus and other Cercopithids on the same section in order to reduce the overall sugar levels and increase fibre levels of their diets. We use the lay meaning of fruit, rather than the botanical one, as this is more readily understandable to those involved in primate feeding. However, we have also reduced the amount of some vegetables that have high sugar levels or removed them completely for the more folivorous species.

Dental health issues declined drastically and stopped completely within a year of the diet change for colobus monkeys. In other groups we saw gradual and sustained weight loss in overweight individuals whilst others in the group maintained healthy weights and we recorded improved faecal consistency and reduced incidences of diarrhoea. This encouraged us to implement fruit free diets across all primates including lorises, lemurs, callitrichids, cebids, gibbons and great apes. We have seen similar physical effects in all these other primate groups. In addition for the lemurs and callitrichids we were able to collect behavioural data. Remarkably consistent results showed reduced aggression and self-directed behaviour (used as indicator of social anxiety) when given fruit-free diets.

Introduction

Although primates are traditionally regarded as relatively easy to feed, there has been growing recognition that inappropriate diets contribute to several common health problems mostly commonly obesity and GIT issues. Traditionally, many primates tend to be regarded as fruit eaters, at least to some extent, and are therefore fed fruit in captivity. This is even the case for some highly folivorous species. Unfortunately, fruit cultivated for human consumption is very different in terms of nutrient composition to leaves and wild fruits eaten by free-living primates. Selective breeding and modern cultivation methods produce fruit that is high in sugars and low in fibre, and therefore high in readily digestible energy. Cultivated fruit also tends to be lower in protein, minerals and vitamins than most foodstuffs consumed by primates in the wild. In addition to contributing to obesity, captive primate diets containing large amounts of cultivated fruit may cause gastrointestinal problems due to low fibre content and poor dental health due to high sugar levels.

Between 2003-2013 we underwent a continual process of review and improvement of diets fed to primates at Paignton Zoo Environmental Park and Newquay Zoo. These were initiated as a result of a number of different issues of concern that could be related to diet, although most of the individuals were generally healthy. The first of these, and the main trigger for diet review, was the poor dental health of several of the Abyssinian *Colobus guereza* and king *C. polykomos* colobus monkeys. Following reviews of these diets, increased awareness of potential nutritional problems, particularly obesity, stimulated keepers to instigate similar reviews of the diets for the rest of the primates across the zoos. Anecdotal reports of behavioural changes following removal of fruit also led us to investigate this in some of the species that were subject to diet reviews later in the process.

Methods

All primates at Paignton Zoo Environmental Park and many at Newquay Zoo have now been included in these diet change investigations. All remained in their usual enclosures with normal husbandry with the only change being diet ingredients (Table 1). All primates are now fed a standard diet of a commercial primate pellet with vegetables (which types varies depending on natural diet) and if appropriate browse, live feeds and/or gum. Cooked wholegrain rice is provided in small quantities to encourage foraging for many species.

Results

The full results related to health and behavioural effects of fruit removal from diets are detailed in published papers (Plowman, 2013; Cabana and Plowman, 2014; Britt *et al*., 2015) but brief results are included for each species in Table 1.

Improved feacal consistency has been reported in most species, even for some species where keepers had not raised it as an issue until they saw improvements and realized what the consistency should really be in those species. For the spider monkeys (*Ateles hybridus*) feacal consistency is still looser than we would like so further review is underway.

Many overweight individuals have lost weight whilst others in the same group have maintained healthy weights without separate feeding. We assume that this is because dominant individuals are no longer motivated to eat more than their “fair share” of the diet since there are no longer any particularly sugary and highly desirable items. Two obese female orang utans have lost considerable weight since 2010 and we had two baby orang utans in 2013 – the first to be born in the zoo since 1998.

The behavioural effects of reduced aggression and self-directed behaviour are remarkably consistent across all species in which we have studied them i.e. all the lemurs and most of the callitrichids. We did not collect formal data on the behaviour of the capuchins (*Cebus xanthosternus*) but keeper notes indicate that one individual performed a pacing/head twisting stereotypy very frequently before the diet was changed. Since fruit was removed from their diets this behaviour has not been noted by keepers.

We have not noted any detrimental effects of fruit-free diets in any species.

Discussion

The results of removing fruit completely from the diets of a range of primates clearly indicate to us that fruit-free diets are beneficial in terms of physical health and behaviour. The physical health effects are most likely due to reduced sugar and increased fibre levels in the overall diet. Other changes of dietary ingredients that have similar effects on those nutrient levels would probably also have similar affects. However, many of the effects on behaviour, particularly aggression and self-directed behaviour, may be more directly related to the absence of fruit rather than the nutrient content of the resulting diet. Removing a highly desirable resource, i.e. sugary, highly palatable food items, may in itself be enough to reduce motivation for aggression and other dominance behaviour in the group.

Removing fruit is an easy, practical method to achieve reduced sugar and higher fibre in primate diets. There are no nutritional benefits of fruit that cannot be acquired in other ingredients so there is no reason to maintain a small amount in the regular diet. Insisting on no fruit at all (using the lay, not botanic definition) rather than still allowing a small amount makes it much easier to prevent keeper drift and ensure the prescribed diets are followed. We only allow small amounts if it is the only way to successfully administer medication. Keepers were initially reluctant to exclude all fruit because they did not want to deprive their animals of their favourtie food items, However, since seeing for themselves the health benefits that have resulted they have become zealous proponents of this strategy and are very willing to spread the message at other zoos.

Literature Cited

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Cabana, F. and Plowman, A.B. 2014. Pygmy slow loris (*Nycticebus pygmaeus*) natural diet replication in captivity. *Endangered Species Res* 23:197-204

Plowman, A. 2013 Diet review and change for monkeys at Paignton Zoo Environmental Park. *J Zoo Aquarium Res* 1:73-77

Table 1. Primate species at Paignton Zoo Environmental Park and Newquay Zoo that are fed fruit-free diets.

Species	Group types	Prior health concerns	Effects of removal of fruit
Nycticebus pygmeaus	Single animals Pairs	Dental condition Overweight Some abnormal behaviour	Improved dental health Gradual weight loss Reduced abnormal behaviour
Lemur catta	Mixed group Bachelor group	Weight management	Reduced aggression and self-directed behaviour
Varecia rubra	Pair	Weight management	Reduced aggression and self-directed behaviour
Varecia variegata	Pair	Weight management	Reduced aggression and self-directed behaviour
Eulemur coronatus	Pair plus young	Weight management	Reduced aggression and self-directed behaviour
Callimico goeldii	Family group		Reduced aggression Increased foraging and active behaviour
Callithrix pygmaeus	Family group		
Saguinus bicolor	Family group		
Saguinus imperator	Family group		Reduced aggression
Saimiri sciureus	Family group		
Pithecia pithecia	Family group		
Cebus xanthosternus	Bachelor pair	Frequent stereotypic behaviour	No stereotypic now seen
Alouatta caraya	Pair		
Ateles hybridus	Pair	Loose feaces Frequent diarrhoea	Improved feacal consistency, although more improvement needed
Cercocebus torquatus	Family group		
Cercoptihecus diana	Family group	Dominant male overweight	Gradual weight loss in male
Macaca nigra	Large mixed group	Dominant male and females overweight Frequent diarrhoea	Gradual weight loss in all overweight individuals Improved feacal consistency
Papio hamadryas	Very large mixed group		Massive reduction in cost of diet for large group
Mandrillus sphinx	Family group	Weight management issues – some under, some over	Healthy weights in all individuals
Colobus guereza	Mixed group	Dental health	No dental issues within a year of change
Colobus polykomos	Mixed group	Dental health	No dental issues within a year of change
Hylobates lar	Mixed group		
Hylobates muelleri	Single female		
Hylobates pileatus	Pair		
Gorilla gorilla	Bachelor group	Weight management	Increased time to consume
Pongo pygmaeus	Mixed group, fed singly	Two females obese	Gradual weight loss First babies (2) in many years

STRONGER THAN YESTERDAY: NEW NUTRIENT RECOMMENDATIONS FOR LORISINE PRIMATES

Francis Cabana[1,2], Ellen Dierenfeld[3], Giuseppe Donatti[1], K.A.I. Nekaris[1]*

[1]Nocturnal Primate Research Group, Oxford Brookes University, Oxford, OX01 0BP, UK
[2]Paignton Zoo Environmental Park, Paignton, Devon, TQ, UK
[3]Ellen S. Dierenfeld, LLC, St. Louis, MO 63128 USA

Abstract
Asia's slow lorises (*Nycticebus* spp.) are heavily impacted by the illegal wildlife trade in Southeast Asia. Confiscated lorises by Customs officials find themselves in rescue centers with approximately 50% of them having had their lower teeth removed by the poachers and must remain in the rescue centre indefinitely, if they survive. Reintroductions do occur but success is very low. Despite evidence from four species in the wild that lorises largely consume exudates, insects and nectar, captive lorises are often fed a high fruit diet, with approximately 63% of facilities reporting diet-related health issues including dental, renal, facial problems, obesity and impaired breeding. The Javan slow loris (*Nycticebus javanicus)* is now critically endangered because of the pet trade and declining habitat. Understand their feeding ecology is essential to determining what they should be fed in captivity. We followed radio collared free-ranging Javan slow lorises (*N. javanicus*) (n = 17) for 10 months at a field site in Cipaganti (Java, Indonesia), itemising and quantifying their native diet and nutrient intake using various methods. Samples of food items were collected for chemical analysis. The food transit and mean retention time trials across five *Nycticebus* species show that the Javan slow loris is a suitable model for the genus and that their morphology and physiology is indeed adapted to digest structural carbohydrates. The current captive diet is far removed from the wild diet in terms of nutrients. The inclusion of gum into the diet increased micronutrient profiles considerably. The new diet trial removed all fruit and included vegetables, gum arabic and insects which created a nutrient profiles similar to wild intake. The new diet had a nutrient profile and food transit and mean retention time similar to wild type diets. This diet is believed to generate future health benefits as well as optimising physiological functions within *Nycticebus* primates.

Introduction
Eight species of slow lorises (*Nycticebus spp*.), small nocturnal primates, are distributed throughout Southeast Asia. A high demand for lorises as pets, photo props and traditional medicine makes illegal trade their greatest threat. To combat this problem, CITES, in 2007 placed *Nycticebus* spp. on Appendix 1, banning all international commercial trade. With a six month gestation leading to litters of one or two infants that require three to six months for weaning, their extremely slow life history does not lend well to this level of off-take (Fitch-Snyder and Ehrlich, 2003).

When enforcement does occur, the most likely destination for slow lorises in Indonesia is a rescue center. As rescue centers reach capacity, personnel investigate the possibilities of reintroduction. Unfortunately the success rate of reintroduction projects are low, with many reintroduced animals succumbing to predation or starvation, not adapting back to life in the wild, or simply unknown outcomes due to lack of follow-up (Soorae, 2008). For slow lorises, numerous newspaper articles report 'success' of individuals simply set free with no follow up. Indeed, only four systematic reintroduction studies are available (Streicher & Nadler 2003; Collins et al 2008). Of 51 confiscated pygmy slow loris (*Nycticebus pygmaeus*) in Vietnam, 15 (29%) died, eleven of which were juveniles (Streicher, 2005). In Java, of 180 confiscated lorises, 64% had their teeth removed. Of those 180, 61 (34%) died despite veterinary care; of 19 reintroduced animals, 90% died (Moore, 2012). Confiscated shipments of lorises show that what we see in markets is the tip of the iceberg. For example, of 102 pygmy lorises confiscated at a Taiwanese airport, > 80% died between confiscation and arrival at their final destination at Saigon Zoo.

Confiscated lorises number in the hundreds in Asia's rescue centers and are fed diets high in fruits, and include dairy, chicken and/or insects similar to diets fed to lorises found in western zoos (Fitch-Snyder et al., 2001; Cabana, 2014). Health issues such as dental problems, facial abscesses, obesity, renal impairment and impaired breeding are rampant in all captive populations (Debyser, 1995; Fitch-Snyder et al., 2001; Cabana, 2014; Fuller et al., 2013). Gum, nectar and insects form the majority of the wild loris diets, albeit minor differences occur in proportions for each species (Nekaris, 2014); no nutritional recommendations are available for any loris species, and current diets are based on anecdotes. There is a clear need to review feeding practices to cater for lorises' physiologic, morphological and behavioral needs if reintroductions are to become successful in their purpose of restoring dwindling wild populations (Kaumanns et al., 2000). This study aims to itemize and quantify both the food items and nutrients ingested by free-ranging critically endangered Javan slow lorises (*N. javanicus*) and to use the Javan slow loris as a model for the creation and validation of lorisine nutrient recommendations through a series of diet trials and validation methods.

Materials and Methods

Wild Data

We were based at the Little Fireface Project's (LFP) field station in the village of Cipaganti, West Java, on the volcano Mount Papandayan (7°16'44.30"S, 107°46'7.80"E, 1200 m ASL). The study area consisted of agriculture fields with interspersed tree and bamboo patches. We followed a group of collared free-ranging lorises (n = 17) using radio collars (BioTrak, UK) and a telemetry antenna and Sika receiver. They were observed six nights/week for a 12 month period with red next generation LED head lamps (CluLite Ltd., UK) and binoculars. Four lorises were also fitted with Activity logging collars (CamNtech, USA) which recorded activity bouts every minute for three months. Behaviors were recorded by instantaneous sampling and food intake was recorded continuously. Table 1 depicts how each food item quantity was measured during intake. Insects were recorded in small (smaller than hand), medium (larger than one hand but smaller than two) and large sizes (larger than two hands). After any fruit feeding occurred, we collected what was left and weighed it using a portable scale (AVI, UK) and compared this weight against a mean value for this fruit to determine weight of eaten portion. Any other food items not planned for was recorded as appropriate to the situation.

Table 1. Observations of food items consumed by slow lorises (n = 17) in Indonesia.

Food Item Category	Recording units
Gum	Seconds (s) of feeding
Nectar	Frequency (#) of flowers
Fruits	Weight of eaten portion
Insects	Frequency (#) of insects
Other	As appropriate

Five 10x10m phenology plots were established in random locations within the field site. Every 1st of the month phenological information was recorded as per Chapman et al. (2005). Presence of gum, mature leaves, young leaves, fruits in tree, fruits on ground and flowers were graded: 0 absent, 1 present but less than 60% of trees capacity, 2 present but more than 60% of tree's capacity. Insect Malaise traps were used in these phenology plots twice/week to calculate insect availability and to weigh a mean value for the different created categories of insects (small, medium, large).

Food Preference

Food preference was calculated by providing a rank to the food items eaten the most to least every month, and ranking the availability of each food resource from most to least. Gum availability did not change throughout the year, so gum tree density was used for its availability every month. These rankings were used in Ivlev's selectivity index (Ivlev, 1961). This equation has been validated by Lehowicz (1982) and used to assign food preference scores in Watts (1984), Vedder (1989), Johnson (1980) and Ganas et al. (2008). Nutrient intake and nutrient proportions of natural food items were plotted on a graph using the Right-angled mixture triangle (RMT) method (Johnson et al., 2013; Raubenheimer, 2011) to determine how each food item contributes to wild diets.

Chemical Composition of Wild and Captive Foods

Samples of the wild diet components were collected and dried in indirect sunlight for 12 hours, then placed into a plastic zip lock bag with silica gel for a maximum of one week before being sent for analysis. Some samples were collected imminently before departure for nutrients which are better detected from fresh matter. A small portion of fresh samples were collected the day prior to going to the laboratory for specific nutrients which require immediate assay. The samples were processed in the same way as was observed eaten by the wild lorises; only ingested plant parts were analyzed. Assays were performed at the Lembaga Ilmu Pengetahuan Indonesia (Indonesian Institute of Science, also known as LIPI) in Bogor by Cabana and the LIPI staff. Gross energy, ash, crude protein, crude fat, simple sugars, soluble fiber, ADF, NDF, Ca, P, K, Na, Se, Fe, Vitamin A, Vitamin D and Vitamin E were all analyzed for wild food items as well as food items consumed in the rescue center. Chemical analysis methods are available in Appendix 1. Collecting and drying nectar in sufficient quantities was not possible, so the only sugar content was determined by using a portable hand held refractometer as per (Bolten et al. 1979); other nutrients were assumed to be present in negligible quantities. To estimate quantity of nectar per flower, 85 microlitre capillary tubes were used to estimate quantity based on volume of nectar collected within tube per flower (Morrant et al., 2009). We measured 100 inflorescences from 5 different plants, totaling 150 flowers over the course of 5 nights.

Captive Trials

After the field season, we collected data at Cikananga Wildlife and Rescue Centre, West Java for 12 weeks. The study population included 12 *N. javanicus*, 4 *N. menegensis* (Bornean slow loris) and 15 *N. coucang* (greater slow loris). All food items used at the rescue center were also sent for chemical analysis. Some individuals either had full, partial or no teeth however, this was not divulged to the researchers until after data collection, and was used as a variable for statistical analysis. At the center, the diet was separated into three meals. Menu 1 was presented at 1900 hr, menu 2 at 2300 hr and menu 3 at 0200 hr. Menus 1 and 2 consisted of one fruit portion and one insect portion each, sometimes with a hardboiled egg, and menu 3 was either more fruit or honey. Diets were evaluated as total daily intake.

Gum Intake Rate

One kg of wild gum from Cipaganti was collected and brought to the rescue centre. Gum intake rate was determined by feeding a known quantity of gum to *N. javanicus* individuals housed alone and using a stopwatch to time total consumption, subtracting any pauses during processing. Conversion (g gum/sec) was used to convert the wild gum intake into gram estimates.

Validation Methods: Intake and Food Mean Retention and Transit Time

The wild feeding data were converted into weights (g) using previously detailed methods. Food intake rates were calculated for each individual as a measure of g/hour on an as-fed (AF) basis. This was used in conjunction with the nutritional information for each food item previously analyzed to create a nutrient intake per hour metric for the Javan slow lorises. Nutrient intake per hour was adjusted by active periods (from logger data) to quantify daily nutrient intake.

Intake studies were conducted with the captive lorises fed their current diet as baseline data for seven days. Daily average amounts, per individual, of the original diets are: katydids (3.4g), Peeled oranges (18.3g), peeled banana (44.0g), mealworms (4.9g), crickets (1.3g), peeled rambutans (12.2g), hardboiled chicken egg without shell (2.2g), sapodilla without seeds (17.1g), honey (4.0g), mangusteen (12.9g) and pine beetle larvae (2.1g). Weights of food items offered and uneaten food removed from the enclosure were weighed. Dessication dishes of food items were also set up and measured at feeding time and at time of clean-up. Feces were collected every day at clean-up time (1000 hr) and individual species' feces were pooled to ensure adequate quantities for chemical analysis to determine apparent digestibility as per Graffam et al. (1998). The mean food retention time was calculated using a non-digestible marker that was fed immediately prior to intake/digestion trials (Lambert, 2002). Coloured plastic beads were used at first without success. The slow lorises were able to use their sublinguals to filter them out and push them out of their mouths. Instead, 1/8 tsp (0.1g) of glitter was mixed into a quarter of a guava fruit (60 g), per individual. The time of first appearance until last appearance was recorded for the glitter and the guava seeds. Four repeats per animal were conducted. Transit time and mean retention times (MRT) were recorded or calculated. A "wild type" diet was fed to the *N. javanicus* (n = 12) for 7 days (with a 25% diet change over 6 days prior to allow for acclimatization) using the proportions of wild food items observed ingested. Intake and transit and food MRT measurements were repeated on the "wild type" diet. MRT trials were also conducted on 2 pygmy slow loris (*N. pygmaeus)* at Paignton Zoo Environmental Park (UK) and on 2 Bengal slow lorises (*N. bengalensis*) at Shaldon Wildlife Trust (UK) for comparisons.

One new diet was created using the wild nutrients ingested as a framework. Only food items which were affordable and available for rescue centres, and were deemed morphologically and behaviorally appropriate for slow lorises, were used. Each individual loris was fed this diet for 14 days (with 25% diet change over 6 days prior to allow for acclimatization). Intake, transit and MRT were measured for this diet to assess if appeared more similar to the wild type diet, which we presume as our “gold standard”. The same validation methods were compared against the original captive diet and the three new diets to determine which appeared the most physiologically appropriate. The average daily new diet, as offered, consisted of mealworms (2.6g). crickets (6.9g), hardboiled chicken egg with shell (1.3g), palm beetle larvae, pupae and adult mix (6.5g), sweet potato (8g), peeled, semi boiled cassava (6.8g), green beans (9.7g), semi-boiled carrots (2g) and gum arabic (10g made with 2:1 parts powder to water).

Results

Wild Feeding Ecology

Wild *N. javanicus* were observed for 256 days for 1470 hours (5.8 hours/night on average) over 12 months totalling 7191 points of data. The unpredictable weather - namely rain and fog - made many nights impossible to find or observe any specimens. Using instantaneous focal sampling, the tally of each feeding observation for each individual was combined and averaged over the total number of data points to represent time spent feeding on different food items and is depicted in Figure 1. These data change substantially when the food items become weighted, and represent actual intake. Figure 2 shows that gum is not the item consumed in the largest amount.

Figure 1. Proportion of time spent consuming different food categories by free-ranging Javan slow loris *(Nycticebus javanicus; n* = 17*)* from instantaneous observations, indicating that almost half of the time is spent feeding on gum.

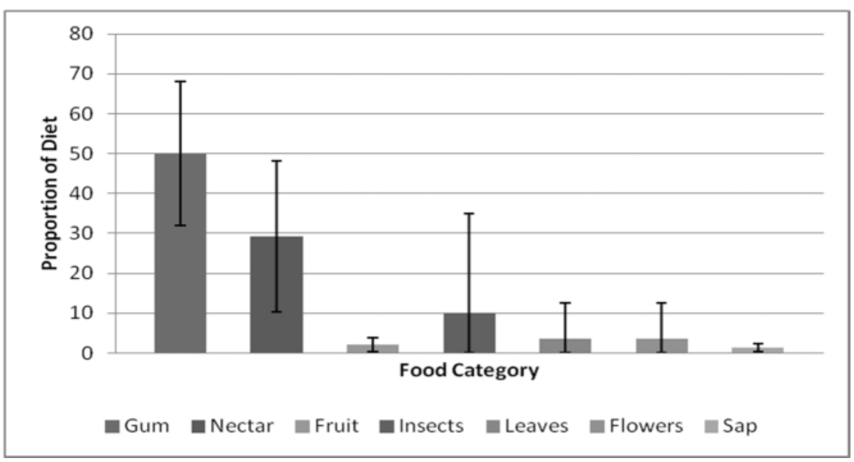
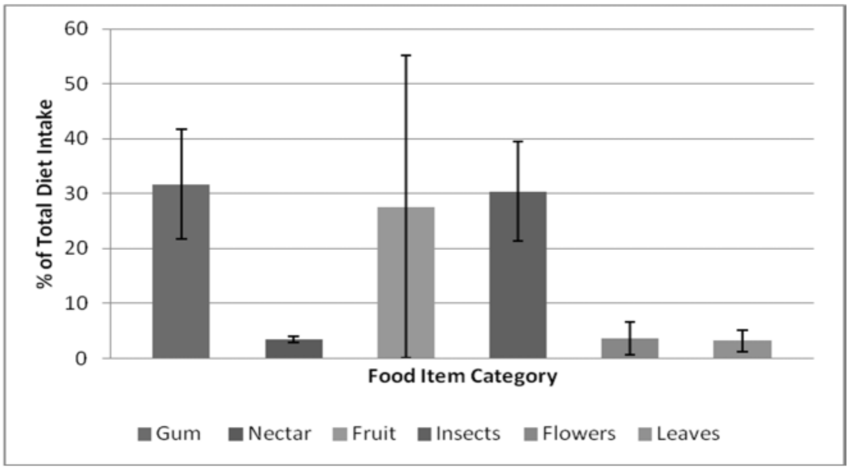


Figure 2. Proportion of actual intake (g as-fed) of different food categories consumed by free-ranging Javan slow loris, (*Nycticebus javanicus*; *n* = 17) according to average intakes measured over 12 months.



Each main staple food item was obtained from one or two plant species. Gum was from an Australian acacia tree, *Acacia decurens*, nectar from Caliandra (*Caliandra catothyrsus)*, fruits from jackfruit (*Arctocarpus heterophyllus*), and persimmon (*Diospyros kaki*), flowers from eucalyptus (*Eucalyptus spp*.) and leaves from bamboo (*Gigantochloa cf. ater*).

Nectar and Gum Intake Rates
One hundred total *Caliandra catothyrsus* inflorescences were sampled totaling 150 flowers. The average amount of nectar obtained from each flower was 22.55 (SD ±1.82) µL and the average sugar content was 22.82 (SD ±5.12) Brix which equates to 253 g of sugar per L of nectar, which we estimate to be 99% of DM and 22.55% as fed (AF).

Gum intake rate was calculated from sampling 12 captive Javan slow lorises which were given 10g of gum harvested from *Acacia decurrens.* On average they consumed gum at the rate of 0.021 g/s. SD ±0.006.

***Food Preferenc*e**
The only food item which had a relatively high preference score (>0.35) was young bamboo leaves as seen in Table 2. Food items which were always available in the same amounts, such as *A. decurrens* gum, resulted in low preference scores.

Table 2: Average values of importance of each food item in the overall diet of free-ranging Javan slow lorises (*N. javanicus; n* = 17), availability of these food items at the field site, and average food preference scores.

Plant Species	Part eaten	Yearly diet proportion (%)*	Avg. Diet rank (SD)	Avg. Avail rank (SD)	# Months observed consumed	Avg pref. score
Acacia decurrens	Gum	31.7	2.18(0.75)	5.33(0.89)	12	-0.42
Calliandra catothyrsus	Nectar	3.4	3.36(1.28)	4.25(1.55)	12	-0.15
Actocarpus heterophyllus	Fruit Pith	6.0	1(0.0)	3.5(0.67)	1	-0.5
Various Insects	Whole	30.4	2(0.89)	1.58(0.66)	12	0.09
Gigantochloa cf. ater	Young Leaf	3.2	4.5(0.58)	2.17(0.83)	4	0.57
Eucalyptus spp.	Flower	3.7	3.25(0.95)	6.17(0.83)	4	-0.33
Diospyros kaki	Fruit Pith	21.7	1(0.0)	5(2.55)	3	0

*Yearly diet proportion is based on intake rates of weighted values, # months refer to the number of calendar months that food was observed being consumed. Average preference scores between 0 and 1 show a preference, while values between 0 and -1 show no preference.

Activity Loggers

Activity logger results clearly delineated active from inactive periods. On average (*n* = 4) Javan slow lorises were active for 11 hours and 54 minutes per day. All 4 animals became active slightly before sunset, and were active only for a brief period of time after returning to their sleep sites before sunrise.

Chemical Composition of Food Items

The chemical composition of wild and captive food items can be found in Appendix 1.

Nutrient Intake

Average intake rate per night (using the 12 hour active period as determined above) of wild *N. javanicus* diet is listed in Table 3. Using the chemical compositions found within Table A1 and the proportional intake of Table 3, we calculated the average nutrients ingested by *N. javanicus* over a 12 month period (Table 4). The RMT is represented in Figure 3, and plots the proportions of NDF, crude fat and crude protein of wild food items as well as wild nutrient intake. Natural food values scaled tightly along the implicit axis (y =-1.0978x + 98.796) which represents crude fat, showing more variation between crude protein and NDF. Results of the captive intake studies for the usual captive diets, and the same diet but with the addition of wild gum, are reported in Table 5.

Table 3: Average food intake rates of free-ranging Javan slow lorises (*Nycticebus javanicus; n* = 17) over 12 months during daily 12-hour activity periods, showing the importance of gum and insects.

Food Category	Gum	Nectar	Fruit	Small Insect	Medium Insect	Large Insect	Flowers	Leaves
Intake rate g/night	10.90	1.17	9.52	0.34	3.08	7.03	1.27	1.11

Table 4: Average daily nutrient intake of Javan slow loris (*N. javanicus; n* = 17*)* with a diet consisting mainly of gum, insects and non cultivated fruits, calculated over 12 months with 12-hour daily activity periods, on a DM basis.

Nutrient	Concentration (DM basis)	Nutrient	Concentration (DM basis)
Energy (Kcal/g)	3.51	Ca:P (Ratio)	2.27
Crude Protein (%)	26.50	Cu (mg/kg)	15.93
Crude Fat (%)	3.38	Fe (mg/kg)	63.34
Soluble Fiber (%)	4.20	Mg (%)	0.10
ADF (%)	11.67	Na (%)	3.69
NDF (%)	18.44	Vit A (IU A/g)	0.72*
Ash (%)	3.74	Vit D (IU A/g)	0.30*
Ca (%)	0.59	Vit E (mg/kg)	1.01*
P (%)	0.26	Soluble Sugars (%)	3.33

*Values not representative of actual intake at time of writing.

Energy value presented above is gross energy; ADF = acid detergent fibre, NDF = neutral detergent fibre

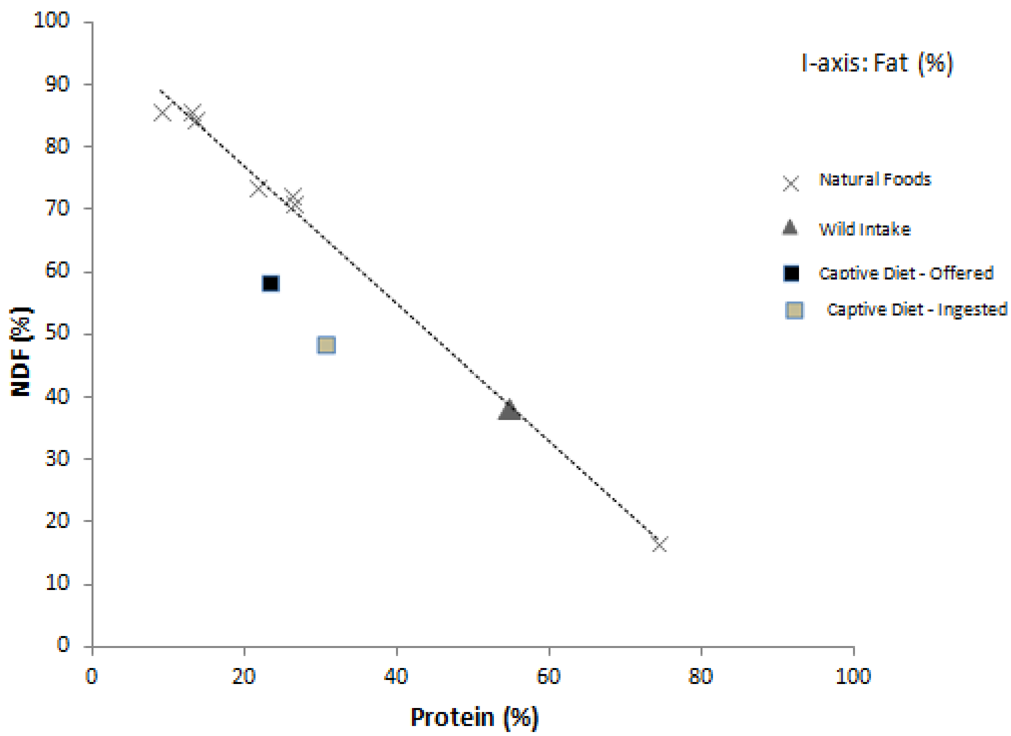


Figure 3 Right-angled mixture triangle (RMT) showing the relative proportions of NDF vs protein vs fat of *N. javanicus* food items (Xs) and the average nutrient intake of free-ranging specimens (triangle). Crude fat contribution is the implicit axis. The dashed line shows the linear regression of natural food compositions (y=-1.0978 + 98.769). The average contributions of crude protein vs NDF vs crude fat of the original captive diets as offered (black square) and as ingested (grey square) are also graphed.

Table 5 Nutrient intake of captive slow lorises (*n* = 31 from 3 *Ncyticebus* spp.) fed typical captive diets comprising fruits, insects and egg, with or without the addition of wild Acacia gum. All data presented on a DM basis.

Nutrient	N. Javanicus	N. Javanicus w/ gum	N. Coucang	N. Menegensis	New Diet N. Javanicus	New Diet N. Coucang
Ash (%)	2.9	2.68	3.11	2.88	3.78	4.03
Crude Protein (%)	12.79	17.46	12.11	13.69	24.38	26.59
Crude Fat (%)	7.58	9.71	7.81	8.3	5.96	6.71
Energy (Kcal/g)	3.92	3.93	3.91	4.25	3.34	3.56
Soluble fibre (%)	0.72*	2.11*	0.71*	0.78*	0.59*	0.61*
ADF (%)	12.28	10.51	24.35	8.41	1.21*	0.78*
NDF (%)	20.56	20.04	14.31	17.3	7.6*	6.15*
Sugars (%)	9.6	6.92	9.2	9.14	0.11*	0.13*
Ca (%)	0.17	0.27	0.14	0.15	0.52	0.53
P(%)	0.19	0.24	0.16	0.2	0.36	0.41
Mg (%)	0.27	0.23	0.29	0.24	0.25	0.16
Fe (mg/kg)	59.47	43.52	57.26	69.12	53.42	50.35
Na (%)	0.43	0.22	0.36	0.12	0.16	0.18
Cu (mg/kg)	7.45	8	6.96	0.2	7.17	7.64
Ca:P	0.89	1.12	0.88	0.75	1.44	1.29

Energy values are for gross energy
ADF = Acid detergent fibre, NDF = neutral detergent fibre
*Chemical analysis results not fully yet obtained so values are represented by less than 80% of diet (DM) at time of writing.

Food Mean Retention and Transit Times

Results of mean retention and transit times are presented in Table 6. All species of *Nycticebus* had comparable transit and retention times.

Table 6: Food transit and mean retention times of *Nycticebus* species held in captivity fed a typical captive diet and for some species, also when fed a naturalistic diet comprising only gum, insects and nectar.

	Diet Time	**# of trials**	***Javanicus*** ***n* = 15**	***Coucang*** ***n* = 15**	***Menegensis*** ***n* = 4**	***Pygmaeus*** ***n* = 2**	***Bengalensis*** ***n* = 2**
Transit Time (hours)	Original Captive Diet (range)	4	25.6 (23.0-31.5)	25.00 (21.5-29.0)	24.2 (21.0-27.5)	29.0 (27.0-31.0)	
	Wild (range)	4	25.6 (24.0 - 29.0)	24.4 (24.0 - 26.5)	24.5 (22.5- 27.0)		
	New Captive Diet (range)	3	25.1 (23.0 - 28.8)	24.7 (22.0 - 28.3)			
Mean Retention Time (hours)	Original Captive Diet (range)	4	51.7 (51.0-52.5)	48.00 (47.0-49.5)	51.1 (48.0-53.4)	58.0 (56.5-59.5)	42.6 (42.8-42.5)
	Wild (range)	4	57.8 (54.5-59.0)	56.3 (54.0-57.5)	52.4 (50.0-54.8)		
	New Captive Diet (range)	3	56.7 (54.0-58.3)	55.9 (53.0-57.0)			
Average weight (g)			1050	936	902	423	1020

Transit time is the first appearance of the marker and MRT is the time until marker is no longer in feces.

Discussion

Wild Feeding Ecology

After observing wild *N. javanicus* for 12 months in an Indonesian agro forest, we were able to create a list of all of their food items. Unsurprisingly, their diets consisted of gum, insects and nectar, which is similar to the diet observed as consumed by *N. pygmaeus* (Starr and Nekaris, 2011). Due to our rigorous and detailed sampling methods of feeding behaviors, as well as excellent visibility, we may have been able to observe a more coherent and complete diet profile than other field sites, which focus on instantaneous data sampling. We were also able to see our specimens ingesting fruit, young bamboo leaves, flowers and sap. *Nycticebus coucang* has been described as spending the majority of its foraging time consuming fruit and nectar (Wiens, 2002). The only other slow loris which has been observed eating leaves as part of its diet is the largest of the genus, *Nycticebus bengalensis* (Das, 2014). It is hypothesised that due to their large size (up to 2 kg), *N. bengalensis* have a larger potential for hindgut fermentation and can exploit the energy of plant parts more effectively (Das, 2014). Without leaves, the large body size would also mean that they would have to catch many more insects to provide enough energy to support their body mass, which may not be sustainable. *N. javanicus* has an intermediate body size within their genus (up to 1.2 kg); omnivory and consumption of multiple plant parts such as bamboo leaves and eucalyptus flowers may not be unexpected, and also suggests this species may be capable of hindgut fermentation.

Food Preference

Our field site experiences one dry and one wet season evenly split throughout the year. Food abundance was expected to show a severe seasonality as seen in other tropical areas with only two seasons (Bearder and Martin, 1980; Chapman and Chapman, 1999). Actual phenological data showed that only fruits and flowers were seasonal, and to a lesser extent, bamboo leaves. Only bamboo leaves were shown to be preferred. The lorises were not observed taking advantage of the abundance of persimmon fruits during the dry season. Gum was a staple part of the diet and available all year round. The gum from *A. decurrens* was shown to have moderate amounts of many minerals, notably calcium, copper, iron and potassium which could help to balance out the high intake of phosphorus found in insects (as in Bearder and Martin 1980). Tree gum was also a source of presumed vitamin A precursors. Overall, the diet of the *N. javanicus* was high in macro nutrients such as crude fat, crude protein and structural carbohydrates. Total dietary calcium to phosphorous ratio was 1.24:1 which indicates that although loris consume a high proportion of insects, they are still able to select other foods that maintain a positive Ca:P ratio.

All of the food items ingested by free ranging *N. javanicus* were rather consistent in their low fat content (~3% of DM, ranging from <1 to a maximum of about 8%), and instead varied widely in fiber and protein (Figure 3). The majority of wild food items appear to be moderate to high in NDF (10 to 65 %) relative to lower protein content (4 to 12%) The opposite is true for the least-consumed food item(s) which are the average values for insects (NDF 14%, crude protein 64%). The insect values are clearly complimentary to the other food items as they are both found on the same axis, on either side of the nutrient intake proportions. The crude fat contents of the wild insects eaten by slow lorises are also relatively low compared with common domestically-reared substitutes (7.72% versus 28.1% for crickets and 27.9% for mealworms on a DM basis). The high prevalence of insects in loris diets may make duplication in captivity difficult. The preferred young bamboo leaves contain the highest NDF proportions (40 to 65% DM), yet do not stand out within the uppermost plant food item cluster; therefore their preference must be due to some micronutrient, possibly.

Captive Trials

Typical captive slow loris diets include domestic fruits and a small amount of a limited number of species of insects (Fitch-Snyder et al., 2001; Cabana, 2014). Zoo diets often include concentrate feeds; less than half of zoos surveyed provide gum, and almost none provide nectar, both of which were found to be staples in wild diets of lorises. These discrepancies lead to diets very rich in protein, soluble carbohydrates and microminerals in zoos (Cabana, 2014) and, as seen in this study, diets which can be nutritionally imbalanced in rescue centers. Lower protein amounts may be adequate for maintenance in captive settings, but the dependence on insects for protein in captivity creates an artificial increase in crude fat and phosphorus, which increases the need for a calcium supplement to balance the negative Ca:P ratio. The addition of gum to the captive diets increased the micronutrient amounts, as well as reversed the negative Ca:P ratio, and increased fibre fractions. The gum was found to be palatable, and even individual lorises which were described as "finicky" by the keepers ingested it readily. Addition of the gum in appreciable amounts (10 g/individual) was not sufficient to raise the nutrient concentrations to identical levels as seen in wild diets; however, it is believed that captive animals will have lower requirements in captivity than in the wild, due to less important physiological stressors and abiotic variations. A number of slow lorises in the rescue center, not used in this study, suffer from metabolic bone disease and dental disease, therefore the current high fruit diet is believed to be inadequate and unbalanced. Figure 3 shows exactly how misplaced and faraway current feeding practices are from wild intake. Zoo loris populations suffer from obesity, dental and kidney diseases as well as inferior quality pelages, thus diets appear inadequate in these instances as well. Diets in rescue centers seem to be too low in quality, and diets in zoos seem to be too rich in specific macronutrients which may all lead to diseases (Cabana, 2014). All captive diets seem to have at least one thing in common, - high soluble carbohydrate concentration owing to excessive fruit proportions. Nectar is the only significant source of soluble carbohydrates in the diets of free-ranging *N. javanicus,* but because nectar is found in such small amounts per flower, it does not translate into a high intake of sugars. Fruits do contain sugars and starches in appreciable amounts, however the wild fruits consumed also contain high amounts of fiber (see Appendix 1) and are not comparable to the cultivated fruits used in captive diets (Schwitzer and Oftedal, 1996; Dierenfeld et al., 2002; Plowman, 2014; Plowman et al., 2015).

Food Transit and Mean Retention Time

The food transit time for *Nycticebus* on a typical captive diet was more or less 25 hours, and did not show a significant difference between species despite large differences in body size. This transit time is much longer than other gummivorous primate groups, the marmosets (*Callithrix:* 3.2 hours and *Cebuella:* 6.3 hours) (Power, 1996). The mean retention time of slow lorises was surprisingly around 50 hours. This would indicate a very high potential for slow lorises to ferment soluble fibres found in gums, and perhaps even exert chitinolytic activity on the ingested insect chitin. The long passage rate of the slow lorises could inherently be caused by their slow metabolic rates, which could then also contribute to fermentation by their gut microflora. Food items should therefore be prioritised by their fibrous components, rather than sugar or starches as once considered. All species studied had very similar transit and mean retention times, which allows us to use the transit and MRT as validation values for the nutrient recommendation diet trials.

Suitability of Javan Slow Loris as a Model Species

The food transit and MRT of different loris species were not significantly different than the wild *N. javanicus* observed. Their digestive systems, feeding ecologies, and presumably physiology are also clearly similar. Captive diet intakes were not dissimilar across species, providing support to our claim that *N. javanicus* can be used as a model for all *Nycticebus spp.,* and that nutrient and feeding recommendations apply widely across this genus.

Slow lorises are known for their low metabolic rates. Muller (1979) successfully illustrated that *N. coucang* had a basal metabolic rate (BMR) of about half that of a primate of the same size , and assumed applicable to all other members of this genus. This observation, combined with Hayssen and Lacy's estimate of BMR (1985), calculates to a mean basal energy requirement of $0.155(W)^{0.755}$ kcal per day, where W is the animal's weight in grams. Using published average wild weights and the equation above, we would estimate the daily BMRs as 28.3, 24.9 and 24.1 kcal/day for *N. javanicus, N. coucang* and *N. menegensis* respectively. These minor differences in energy requirements may be met using identical diets differing only in total mass offered/consumed.

With the majority of captive *Nycticebus* diets containing high amounts of fruit and low amounts of exudates, current diets are not representative of slow loris feeding ecology adaptations

(Cabana, 2014). Their digestive physiology and morphology are rather adapted to consume and exploit large amounts of exudates (Nekaris, 2014). It may be impossible for many centers to recreate these wild diets and their chemical and physical characteristics. Here we demonstrate that the removal of fruit and honey, and the regular inclusion of vegetables, gum Arabic and insects to the diet altered the nutrient profile to better reflect wild nutrient intake, and provide a more beneficial (nutritionally balanced) and positive physiologically functioning diet.

Conclusion

According to available results from this study, the Javan slow loris (*Nycticebus javanicus*) is a suitable model for the genus. Food transit and mean retention times, behavioural profiles and intake rates were similar for all species. This study is the first to describe nutritional profiles of foods eaten by free-ranging *Nycticebus* spp., and well as transit and food mean retention times of *Nycticebus* species held in captivity. We translated one year's worth of wild *N. javanicus* observations into nutrient recommendations that led to generalised captive feeding guidelines. Future research should focus on how changing the nutrient profile of the captive loris diets affects their health and the propensity of diseases long term.

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APPLE LEAVES AND BARK AS BROWSE ITEMS FOR HEBIVOROUS MONO-GASTRICS UNDER HUMAN CARE

Sarra Gourlie[1,2]*, Jim Atkinson[2], and Jaap Wensvoort[1]

[1]Toronto Zoo, 361A Old Finch Ave. Toronto, ON, M1B 5K7
[2]University of Guelph, 50 Stone Rd East, Guelph, ON, N1G 2W1

Abstract
Many zoological institutions are faced with challenges when developing and providing a diet for their non-ruminant browsing herbivores. The most prominent issue remains providing readily available, safe and sufficient quantities of plant material. Zoos are often forced, usually by climate, to utilize seasonally available, local, appropriate and or novel plant species in order to decrease the effort and expense required to cultivate or import plant foods naturally consumed by species in the wild. The main goals of this study were to (1) quantify the edible portions of an apple browse branch, (2) provide a framework for evaluating the suitability of apple browse as a safe edible item for non-ruminant herbivores.

It is essential to quantify the contribution of browse to the diet, which includes an understanding of the composition, and the relative proportion of any individual plant part. These parameters can then be utilized to better predict the physiological impact and to enhance diet formulations. Leaves, bark and twigs were analyzed for their nutritive profile periodically over the course of the harvesting period (May to October). Results demonstrated little seasonal effect on nutritive parameters. Apple leaves contained on average the highest level of protein (9.6% DM) compared to bark (4.1% DM) or twigs (3.9% DM). Apple bark and twigs contain high levels of both ADF and NDF relative to leaves. The micronutrient analysis revealed that leaves have the highest level of Fe content. Apple bark contained highest level of Ca and apple twigs the highest level of Zn. Neither leaves, bark nor twigs contained significant levels of Na. Allometric equations reflecting the correlation between basal diameter and plant portions (leaf, twig and bark) were established. The corresponding r^2 values indicated that basal diameter was well correlated with whole branch weight and bark weight; however leaves and twigs did not demonstrate a high correlation.

An animal feeding trial was performed in order to gain insight into the possible physiological effects apple leaves or bark could have upon ingestion by a non-ruminant herbivore. A large feeding trial was performed with 36 young male New Zealand White rabbits. Rabbits were randomly assigned to one of 6 treatment groups. Three treatment groups were offered rabbit pellets at 80% ad-libitum level and were offered no browse (control) or ad-libitum apples leaves or bark. Similarly, the remaining three treatment groups were offered 50% ad-libitum pellet levels and offered no browse (control) or ad-libitum apple leaves or bark. The trial length was 4 weeks in total and blood was collected before and after the trial for serum analysis. AST and ALT levels were found to be within previously published normal physiological ranges, indicating no unusual negative effect of treatment diets. Serum cholesterol, HDL-c and triglyceride concentrations were assessed.

Rabbits in groups consuming low browse and high pellet exhibited highest circulating triglyceride levels. Rabbits consuming highest levels of either apple leaves or bark had the highest ratio of HDL-c:cholesterol. Antioxidant markers glutathione peroxidase (GlutPx) and superoxide dismutase (SOD) were measured. Results indicated no effect on SOD or GlutPx activity. Liver and kidney weights indicated no difference between treatments. Overall results indicate that browse consumption does not negatively affect liver or kidney function, serum antioxidant activity, or organ weights. A high ratio of HDL-c:cholesterol, as seen in the high browse groups, indicates a potential beneficial response to treatment diets. In conclusion, apple browse leaves and bark are a good quality and safe dietary items that imparts little to no risk to mono-gastric herbivores. We can now make better dietary recommendations and formulations using allometric equations and nutritive profiles of plant parts.

CASE STUDY: UNINTENDED CONSEQUENCES OF ADDRESSING GI DISTRESS IN A SIAMANG (*SYMPHALANGUS SYNDACTYLUS*)

Erin Kendrick, MS* and Mike Maslanka, MS

Department of Nutrition Science, Smithsonian Conservation Biology Institute, Washington, DC USA

MEDICAL NUTRITION THERAPY FOR HUMAN GASTROINTESTINAL DISORDERS AND APPLICATION TO CAPTIVE NON-HUMAN PRIMATES

Adam Reppert, MS, RD

Fort Worth Zoo, 1989 Colonial Parkway, Fort Worth, TX 76110

Abstract
Abnormal gastrointestinal function is considered to be a common problem among captive non-human primates. Certain nutrients and diet patterns are implicated in abnormal gastrointestinal function and disease. The human nutrition field uses medical nutrition therapies to prevent and/or manage a variety of gastrointestinal disorders. Some of these may be appropriate for application to non-human primates. Current evidence-based nutrition interventions for gastrointestinal disorders in humans are reviewed, and application to captive non-human primates discussed.

Introduction
Abnormal gastrointestinal (GI) function, ranging from chronic loose stool to diagnosed diseases, is considered a common problem among captive non-human primates. Chronic diarrhea is one of the issues most often reported in literature for many species of apes and monkeys, usually due to an infectious agent, but sometimes of no identifiable etiology (Lewis and Colgin, 2005; Munson and Montali, 1990; Rubio and Hubbard, 2002). For example, diarrhea was found to be the primary diagnosis for 32.5% of the clinical caseload at the Oregon National Primate Research Center (Wilk et al., 2008). Colitis is also a frequent post-mortem finding in animals with non-infectious diarrhea (Lowenstine and Rideout, 2004).

Diet and nutrition are fundamental to gastrointestinal health and function. Plant fiber, for example, positively impacts satiety, fecal consistency and overall GI health in primates (Cummings, 1978; Morin et al 1978; NRC, 2003). Dietary modifications may benefit primates when inappropriate diets are suspected as causal factors in GI abnormalities, although improvements are sometimes inconsistent (Janssen, 1994; Plowman, 2013). In humans, various medical nutrition therapies are used to prevent and manage GI disorders (AND, 2014; Dore et al., 2008; Eiden, 2003; Nahikian-Nelms et al., 2011; Stephen and Gyr, 2004). Some of these therapies may be appropriate or translatable to captive non-human primates with similar GI symptoms or conditions.

Medical Nutrition Therapy (MNT) for Human Gastrointestinal Disorders
Irritable Bowel Syndrome (IBS)
IBS is a chronic functional disorder of the gastrointestinal tract that commonly presents as cramping, abdominal pain, bloating, gas, diarrhea and constipation (Nahikian-Nelms et al., 2011). The most current nutrition therapy for IBS includes individualization along with:

- Low-FODMAP diet (fermentable oligo-,di-, and monosaccharides and polyols) (Ong et al., 2010; Heizer et al., 2009; Shepherd et al., 2008)
- Fiber intake based on tolerance and IBS type (AND, 2014)
- Elimination diet to identify food triggers
- Vitamin/mineral supplementation for suspected deficiencies

- Consideration of pre- and probiotics (Clark et al., 2012)
- Consideration of peppermint oil (Ford et al., 2008)

Inflammatory Bowel Disease (IBD)

IBD, which includes Crohn's Disease and Ulcerative Colitis (UC), involves chronic inflammation (often including ulcerations) in all or part of the intestinal tract, and presents with symptoms such as severe diarrhea, pain, fatigue, and weight loss (Eiden, 2003; Graham and Kandil, 2002). Nutrition therapy differs based on whether the disease is active or in remission.

During active flare-up/exacerbation:

- Bowel rest if needed; supportive nutrition (e.g. enteral or parenteral feeding) may be appropriate
- Progress to low-fat, low-fiber, high-protein, high-calorie, small, frequent meals with return to normal diet as tolerated
- Vitamin/mineral supplementation as needed to correct deficiencies or malabsorption

During remission:

- No particular diet is known to maintain IBD in remission; a normal "diet as tolerated" remains recommended (maintenance diet should strive to meet nutrient needs and support maintenance of a healthy weight) (Eiden, 2003)
- Avoid foods high in oxalate with Crohn's disease (AND, 2014)
- Consider supplementation with n-3 fatty acids and glutamine (Eiden, 2003)
- Consider use of pre- and probiotics (Gassull, 2005; Guarner, 2005)

Celiac Disease (Gluten allergy)/Gluten Intolerance

The only effective therapy for true gluten allergy is a strict gluten-free diet (Fasano and Catassi, 2001). For those suspecting or diagnosed with the less well-defined gluten intolerance, avoidance or minimization of gluten in the diet may provide relief, however, more recent research suggests this population may actually be intolerant to certain FODMAPs, as in IBS (Biesiekierski et al., 2013). Wheat contains both the protein gluten and the carbohydrate fructan, and the latter may be responsible for the symptoms attributed to gluten.

Diverticular Disease

Diverticular disease is characterized by the formation of tiny pockets, called diverticula, in the lining of the large intestine (colon), and includes diverticulosis, diverticular bleeding, and diverticulitis (Nahikian-Nelms et al., 2011). Nutrition therapy differs for diverticulosis and active diverticulitis.

Diverticulosis:

- High-fiber diet (≥16-17 g/1000 kcals/d) (gradual increase to ensure tolerance) (Makola, 2007)
- Avoidance of nuts, seeds, and hulls no longer considered necessary (Strate et al., 2008)
- Consider probiotics (Douglas, 2008)
- Adequate fluid intake in conjunction with high-fiber diet

Diverticulitis:

- Bowel rest (NPO) until bleeding and diarrhea resolve
- Begin oral intake with clear liquids, and slowly advance to low-fiber diet until inflammation and bleeding have fully resolved; then gradually return to high-fiber diet
- Consider probiotics

Gastroesophageal Reflux Disease (GERD) and Peptic Ulcer Disease (PUD)

GERD is a chronic disease in which stomach acid (or occasionally stomach contents) flows back into the esophagus, thereby irritating the lining of the esophagus and causing symptoms such as reflux or heartburn, and over time can cause damage to the esophagus. PUD involves an imbalance in digestive fluids in the stomach and small intestine that result in painful ulcers or sores in the lining of the stomach or duodenum (Shapiro et al., 2007). While the cause of both conditions can vary, dietary factors are often implicated, and the recommended nutritional therapy is similar for both (Dore et al., 2007; Shapiro et al., 2007):

- Avoid foods that can increase gastric acid secretion or damage the gastric mucosa (black and red pepper, coffee, tea, alcohol)
- Smaller, more-frequent meal pattern
- Eliminate/minimize foods that reduce lower esophageal sphincter pressure (chocolate, mint, high-fat foods)
- Lose weight if overweight
- Avoid eating 2-3 hours before bedtime
- Sit upright after meals

Constipation

Constipation is defined as infrequent and/or difficult bowel movements. While normal bowel movement frequency and consistency varies from person to person, a period of more than 3 days between bowel movements is considered abnormal, as is straining during a bowel movement or passing a hard bowel movement more than 25% of the time (Ternant et al., 2007). Nutrition therapy for constipation aims to improve the frequency of bowel movements and prevent straining during a bowel movement, using the following strategies:

- High-fiber diet (gradual increase to ≥25-38 g/d or 14 g/1000 kcals/d) (Ternant et al., 2007)
    - Insoluble fibers (found in whole grains and vegetables) appear to have the greatest effect on resolving constipation, but there is no definitive evidence of ideal fiber type(s); individuals are recommended to try different fiber formulations to find which work best for them (AND, 2014)
- Adequate fluid intake (≥64 oz/day)
- Regular physical activity
- Consider use of bulk-forming agents such as psyllium or methylcellulose (AND, 2014)
- Consider pre- and probiotic-containing foods as part of daily diet (AND, 2014)

Diarrhea

Diarrhea is characterized by loose and watery bowel movements, and can range in severity from acute and benign to chronic or indicative of serious disease, depending on the cause. Treatment of the underlying cause is the most important aspect of diarrhea therapy. Nutrition therapy for diarrhea should aim to achieve the following:

- Restore normal fluid, electrolyte, and acid-base balance first (Guerrant et al., 2001)
- Reduce gastrointestinal motility
    - Avoid foods/beverages high in simple carbohydrates and sugar alcohols (AND, 2014)
    - Avoid high-fiber and gas-producing foods (AND, 2014)
- Improve stool consistency
    - Consider adding sources of pectin such as banana flakes to foods (Duro and Duggan, 2007)
    - Consider trial of coconut flakes or oil (Graedon and Graedon, 1999)
- Repopulate gastrointestinal tract with healthy microflora
    - Probiotic/prebiotic supplementation may assist with treatment and recovery but current research does not substantiate dosage recommendations (Canani et al., 2007; Douglas and Sanders, 2008)
- Stimulate the GI tract with slow introduction of solid food without worsening of symptoms
    - Low-fiber, low-fat, lactose-free foods recommended initially (Duro and Duggan, 2007; Steffen and Gyr, 2004)

Considerations in Application to Non-Human Primates

The aforementioned medical nutrition therapies (MNTs) have fair to strong evidence basis for use in humans (AND, 2014). Given the physiological similarities between many species of non-human primates (NHPs) and humans, and the similar nature of GI disorders observed in both, it may be appropriate to apply these therapies to NHPs in some instances. Before application, however, it is necessary to consider several factors. Each species' feeding ecology and digestive physiology should always be considered. Humans are omnivorous with a relatively simple GI tract, therefore certain nutritional therapies may not be appropriate for NHP species with different digestive strategies, such as foregut-fermenting colobines or hindgut-fermenting howlers (Bauchop and Martucci, 1968; Milton and McBee, 1983; Stevens and Hume, 1995). These highly folivorous primates need to maintain higher levels of plant fiber while minimizing readily fermentable carbohydrates for normal microbial fermentation and GI function (Edwards and Ullrey, 1999). The consequences of inappropriate diets for these species are reported in literature (Bauchop and Martucci, 1968; Janssen, 1994).

Another consideration is the common use of nutritionally-complete feeds in the diets of many captive NHPs. These items may contain ingredients commonly nontolerated by humans with certain GI disorders (e.g. wheat, soy, fructose, fibers), so it is possible these same ingredients may not be tolerated by individual NHPs with similar GI issues. If these fortified diet items are removed as part of therapy, the diet will need to be reassessed to ensure nutritional adequacy. Replacing nutritionally-complete feeds is a challenge in NHPs because there are fewer food groups/substitutions considered appropriate for NHPs from which to choose to meet nutrient needs compared to humans, and natural items (e.g. browse) often are not sufficiently available to supply key nutrients like protein and fiber.

Food preferences of individual animals also may complicate application of nutritional therapies. Animals with limited preferences may not accept replacement diet items or other modifications (e.g. will not consume more high-fiber leafy vegetables). Such animals may also resist novel ingredients like resistant starch and probiotics either mixed into diet items or as stand-alone supplements.

Yet another challenge is the potential difficulty monitoring and evaluating a therapy for efficacy in captive NHPs. This includes an inability to monitor an individual's response if maintained in a group setting (e.g. identifying one animal's stool from that of others), and the presence of confounding factors like stress, infection, and medications that may disguise or counteract any benefit the therapy is providing.

Some non-human primates are also prone to certain GI-related behaviors not seen in humans, for which human MNT may or may not be relevant. One common example is the regurgitation/reingestion behavior most commonly reported in captive gorillas and other apes. This behavior is observed only in captivity, and has been attributed mostly to stress and boredom, but also to certain diet items (Akers and Schildkraut, 1985; Baker and Easley, 1996). Recent research, however, suggests this behavior could also be related to GERD, but may not be recognized as such due to the difficulty diagnosing GERD in NHPs (Glover et al., 2008).

Finally, in deciding whether to apply human MNT for GI disorders or symptoms in NHPs, non-nutrition-related causes for symptoms should be considered. Behavioral dynamics and stress can have significant effects on stool quality and other GI symptoms, as has been documented in NHPs living in group situations (Wilk et al., 2008). Infection should be ruled out as a treatable source of GI dysfunction and dysbiosis. Recently, there is also a growing recognition of the role of obesity in gastrointestinal health and function, warranting consideration of an animal's weight or body condition status as a factor in any GI symptoms or disorders (Clemente et al., 2012; Moayyedi, 2008). These factors are important to recognize because the GI symptoms and conditions described above can occur in the context of an appropriate diet. Assessment of the complete clinical, nutritional, and behavioral situation is needed to properly apply human MNT to non-human primates.

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ASSOCIATIONS BETWEEN DIET, GUT MICROBIAL COMMUNITIES, AND HEALTH IN RED-SHANKED DOUCS (*PYGATHRIX NEMAEUS*): A MODEL FOR THE SUBFAMILY COLOBINAE.

J. B. Clayton[1,2], D. Knights[1], F. Cabana[5]*, H. Huang[1], L. T. Ha[2,4], T. V. Bui[2], M. V. Vo[5], D. A. Travis[1], and T. J. Johnson[1]

[1]University of Minnesota, Saint Paul, MN, USA
[2]GreenViet Biodiversity Conservation Center, Danang, Vietnam
[3]Paignton Zoo Environmental Park, Paignton, UK
[4]Frankfurt Zoological Society, Frankfurt, Germany
[5]Danang University of Education, Danang, Vietnam

Abstract
Red-shanked doucs (*Pygathrix nemaeus*) and other colobines possess specialized gastrointestinal systems similar to ruminants. They utilize both fore- and hindgut fermentation to meet their energetic demands. Maintenance of captive populations has been largely unsuccessful. Improving captive conditions is hindered by critical gaps in our understanding of their natural diet and enteric microbial adaptations that facilitate the digestive process. We used the douc as a model to study the relationships between diet and microbial community activity within the gastrointestinal tract. Fecal samples from 7 wild and 27 captive red-shanked doucs were collected between 2012-2013 from Son Tra NR (Vietnam) and 3 captive facilities. We measured gut microbiome composition using 16S rRNA sequencing. PICRUSt software was used to predict microbial function. Feeding behaviors of wild doucs were surveyed using focal sampling. Foraged plant species were collected and analyzed for nutrient content. Dietary records, including nutritional data, were provided by the captive facilities. Statistical analyses were performed to identify correlations between diet, gut microbiome, and animal status (captive vs. wild). Analysis of similarity revealed that gut microbial communities grouped by animal populations (ANOSIM R = 0.92; P = 0.001). A reduction in gut microbiome richness and diversity was observed in captive doucs. We identified microbial biomarkers of douc nutritional health. We hypothesize that captivity causes doucs to shift to severe gut dysbiosis, thereby resulting in GI issues, malabsorption and failure to thrive.

NUTRITIONALLY COMPLETE FOOD-FREE DIETS FOR PRIMATES: POTENTIAL BENEFITS AND CONCERNS

Barbara A. Henry, MS[1]*, and Adam Reppert, MS, RD[2]

[1]Cincinnati Zoo and Botanical Garden, 3400 Vine St., Cincinnati, OH 45220
[2]Fort Worth Zoo, 1989 Colonial Parkway, Fort Worth, TX 76110

Abstract

Many zoos and institutions offer nutritionally complete foods (NCF) in the diets of captive primates (Oftedal and Allen, 1996). These foods, which often take the form of extruded biscuits, canned diets or gels, provide a source of important nutrients like protein, fat, fiber, vitamins and minerals, thereby helping to meet minimum estimated nutrient requirements (Oftedal and Allen, 1996; NRC, 2003). These nutrients can otherwise be difficult to fully supply with commercially available produce and other items appropriate for nonhuman primates. Additionally, NCF provide an energy and nutrient dense diet item for animals with increased energy or nutrient needs (e.g. for weight gain/underweight, pregnancy/lactation, etc). Furthermore, NCF are cost-effective and operationally easy to procure, store and provide in the diet. These combined benefits address many of the considerations involved in formulation (Crissey, 2005).

Even though NCF provide many nutritional and operational advantages, there are anecdotal and documented concerns about providing them in primate diets (Ball et al., 2008; Less, 2012; Cassella, 2012). They have the potential to provide excess calories and contribute to obesity due to their energy density. However, if diets are formulated appropriately and monitored regularly, they can be adjusted to avoid over-feeding. It is important to examine the ingredients within the products offered and the nutrients they provide (Schmidt et al., 1999). Another concern, which is largely anecdotal, is the perception that NCF might adversely affect gastrointestinal tract function and perhaps stool quality (e.g. contributes to regurgitation, diarrhea/loose stool) (Ball, et al., 2008; Gould and Bres, 1986). Some zoos report individual animals with chronic loose stool, with NCF suspected as contributory; some of these animals seem to respond to removal of the NCF from diet, while others do not, and it is important to consider the multifactorial nature of gastrointestinal abnormalities (Shaw and Rich, 2007). Yet another objection to NCF is the belief that they are not “natural” and thus do not belong in the diet. Other stated concerns include perception that NCF contribute to behavioral problems (e.g. dominance or feeding aggression issues in groups) and that they contain ingredients perceived to be non-ideal for or allergenic to nonhuman primates (e.g. wheat, corn, soy). Though allergies to or intolerances of specific ingredients should be considered, so must nutrient levels in food items and the overall diet. For example, it has been suggested high levels of fat, starch and sugars in the diets of wooly monkeys may lead to metabolic and cardiovascular problems, rather than NCF specifically (Ange-van Heugten et al., 2008). Furthermore, NCF formulations and options have changed over the years, with many primate NCF now available in lower-sugar, lower-starch, and higher-fiber varieties. These confounding factors may contribute to varied responses to diet changes in primates (Ball et al, 2006).

Institutions that decide not to include NCF in their primate diets face several challenges. First and foremost is difficulty meeting minimum nutrient needs, particularly protein, fiber, and some micronutrients, with a related challenge of finding or providing appropriate novel protein sources

(operational challenges, acceptance issues, etc.). Commercially-available produce, for instance, has been found to be much lower in fiber than wild plants and fruits that would be part of free-ranging primate diets (Schmidt, 1999). While browse may be a suitable source of protein and fiber, and should be considered in primate diets whenever possible (Oftedal and Allen, 1996), many zoos are unable to regularly provide this item. Meeting nutrient needs without NCF may be especially challenging in animals with increased and/or special nutrient needs (e.g. underweight, pregnancy/lactation, nutrient deficiencies). Finally, the ability to monitor and assess individual animals to ensure dietary adequacy will be even more important without NCF in the diet.

The benefits and concerns of feeding nutritionally complete foods as part of captive primate diets, and the challenges of formulating diets without them, will be further explored in an interactive discussion session.

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I WANT TO BE A ZOOLOGICAL ANIMAL NUTRITIONIST OF VETERINARIAN BUT HOW DO I AFFORD THE COST OF MY EDUCATION? STUDENT DEBT RELIEF

Amelia Lockhart, JD Candidate, MBA, Finance GC

Evergreen Education Society, 2013 Harkins Street, Bremerton, WA 98310

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AN ANALYSIS OF VITAMIN C SUPPLEMENTATION IN THE DRINKING WATER FOR GIANT ELEPHANT SHREWS (*RHYNCHOCYON PETERSI*) AT THE PHILADELPHIA ZOO

Amanda Christine Steinagel[1], Barbara Toddes[2], Donna M. Ialeggio[2], and Benjamin Golas[3]

[1]The Ohio State University College of Veterinary Medicine, 1900 Coffey Road, Columbus, OH 43210, USA
[2]Philadelphia Zoo, 3400 W. Girard Avenue, Philadelphia PA 19104
[3]Colorado State University, Fort Collins, CO 80523, USA

Abstract

The Philadelphia Zoo acquired 2.2 Giant Elephant Shrews, *Rhynchocyon petersi*, in July 1999 for the purpose of exhibition and reproduction. At that time, limited anecdotal dietary and activity budget data were available for this species. The animals appeared to be clinically healthy and in 'ideal' nutritional condition upon arrival. Intensive observational studies of time budgets and food and water intake were undertaken by Philadelphia Zoo mammal staff.

Over a period of years, a decline in pelage and skin condition in adults was appreciated; several animals were diagnosed with periarticular (primarily) swellings associated with *Mycobacterim avium* sp infection; and multiple cases of hand-reared offspring succumbing to esophageal and/or gastric rupture, and/or abnormal cartilage development resulting in extensive pathologic vertebral fractures were appreciated. Review of dietary, clinical pathology and histopathologic evaluation of morbidity and mortality in hand-reared neonates was strongly suggestive of vitamin C deficiency/insufficiency disease.

Based on a working hypothesis of vitamin C-related morbidity and mortality, supplementation of hand-reared neonates with injectable sodium ascorbate, and oral supplementation of vitamin C in drinking water of juveniles, subadults and adults was initiated, with vitamin C added to drinking water based on documented giant elephant shrew daily water intake and at a level intended to meet the vitamin C requirements of guinea pigs (as a starting point). Following onset of routine vitamin C supplementation of hand-reared neonates, morbidity ascribable to putative vitamin C deficiency in this subset of giant elephant shrews ceased. In addition, improvement in skin and pelage quality of the adults was appreciated. As of this time it has not been established whether this species may have increased requirements for vitamin C more than any other mammal species; nor has it been determined whether giant elephant shrews require exogenous dietary vitamin C.

The Philadelphia Zoo uses reverse osmosis (R/O) water as drinking water for small animals due to the high level of some minerals in city tap water. To evaluate the efficacy of oral vitamin C supplementation in drinking water in this setting, two separate studies were completed. In the first study the availability of vitamin C added to either Philadelphia City tap water or R/O water was evaluated *ex situ*. Measurable levels of vitamin C were found in R/O water but not tap water over the study period. In the second study, samples collected directly from the drinking water offered in two shrew exhibits were evaluated to assess the availability of supplement in drinking water under real-time exhibit conditions. Incidentally, it was discovered that some keepers crushed the half wafer prior to adding it to the drinking water while others added it to the water

as an uncrushed half wafer, both presentations were evaluated. As in the first study, vitamin C activity when offered in R/O water was measurable throughout the sampling period.

Introduction

The important role of ascorbic acid (Vitamin C) has been established for many mammalian species. Ascorbic acid is a water-soluble antioxidant that acts as an enzyme cofactor in bodily functions (Mandl et al., 2009). Vitamin C has been shown to decrease free radical and reactive oxygen species that are created during reactions within the body (Mandl et al., 2009). Ascorbic acid has also been associated with collagen synthesis and maintenance, and has a protective role for the cells that are commonly involved in the immune system (Hidiroglou, 1999).

Vitamin C can be supplemented in multiple ways, including the use of concentrated juices, whole fruits or parts of fruit, and synthetic administration in the feed and/or water. Older publications, such as Hughes and Jones (1971), suggest the addition of natural sources of vitamin C like black currant and acerola cherry for mammalian species. Oranges and orange peel, specifically, have been found to have abundant vitamin C for supplementation (Wilson et al., 1976). More recent studies have suggested that synthetic vitamin C can be used to supplement diets with ascorbic acid. Hidiroglou (1999) proved that synthetic vitamin C tablets can be added to water offered to cattle and this method of supplementation is efficacious and will result in an increase in serum ascorbic acid levels. Water supplemented with vitamin C tablets has also been used in mice to investigate the effects of hypovitaminosis C. Detectable concentrations of vitamin C in the tissue of mice were found after 5 weeks of supplementation (Kim et al. 2012). This same study investigated the effects of removing the vitamin C supplementation after sufficient levels were found. After one week of no vitamin C supplementation in the water, there was a significant decrease in the concentration of vitamin C found within the tissues and serum of the mice (Kim et al., 2012).

Historically, zoos have used these various methods to supplement captive diets with vitamin C for animals known or believed to be at risk for deficiency. However, the addition of synthetic sources of vitamin C, such as vitamin C tablets, to water is commonly believed to not be effective. There are studies that support this belief. For example, Moody et al. (2008) found that guinea pigs supplemented with vitamin C tablets in water, do not have corresponding increases in vitamin C levels in their serum after supplementation.

Humans, non-human primates, guinea pigs, and other guinea pig-like species do not have the ability to produce ascorbate due to a deficiency in gulonolactone oxidase, the last enzyme in the pathway that converts ascorbic acid into the active form of vitamin C, ascorbate (Mandl et al. 2009). Clinical manifestations of hypovitaminosis C have been reported in necropsy findings in Giant Elephant Shrews (GES). These findings most commonly include different types of tissue inflammation, hemorrhage, and necrosis. A gross necropsy report of a giant elephant shrew that was not supplemented with vitamin C showed lesions affecting multiple organ systems. There was chronic moderate pericarditis and acute, bilateral, multifocal hemorrhage located within the lungs. Thoracic hemorrhagic effusion, as well as, abdominal hemorrhagic effusion were both present. Multifocal portal thrombosis, was also noted on gross examination. The integumentary system showed acute, severe, ulcerative dermatitis, as well as, acute, severe, necrotizing cellulitis. The musculoskeletal system showed moderate to severe, chronic synovitis and osteoarthritis of the stifle and tarsal joints, bilaterally (Trupkiewicz, 2007).

The purpose of this publication is to validate the practice of supplementing the R/O drinking water with vitamin C, through analysis, for animals that require vitamin C in their diet. Although necropsies of GES at the Philadelphia Zoo are suggestive of a dietary vitamin C requirement, this was not the goal of the studies and work to conclusively determine a vitamin C requirement in GES has not been done. Two related studies were preformed: 1) tested the viability of vitamin C supplementation in R/O water and tap water, over a 24-hour period and 2) tested the viability of vitamin C supplementation added to R/O water in the form of a half uncrushed wafer and as a crushed half wafer, over a twenty-four hour period in order to determine if there is a difference and the best form for optimal supplementation.

Materials and Methods

Experiment 1

This study was done to determine the activity of vitamin C in Philadelphia tap water versus R/O water. Water was not in the animal exhibits.

Ascorbic Acid Supplementation

125 mg ascorbic acid (1/2 wafer, Tangy orange flavor, Natural Factors), crushed with a mortar and pestle, was added to 500 mL of either reverse osmosis (R/O) water or tap water, the water was sampled at 0, 12 and 24 hour intervals to determine how the concentration of vitamin C changed over 24-hours and if water type made a difference. A container of each supplemented water type was held under conditions mimicking the temperature, light, and humidity levels of the GES exhibits. Water samples (10mL) were collected from each container at each interval. Samples were wrapped in aluminum foil, frozen at -40 ^{o}F, and submitted on dry ice to Midwest Laboratory for determination of the amount of vitamin C present.

Experiment 2

This study was done to ensure the same results under exhibit conditions with vitamin C supplemented R/O drinking water and to determine if there is a difference in the C activity between crushed versus uncrushed half wafers

Ascorbic Acid Supplementation

125 mg ascorbic acid (1/2 wafer Tangy orange flavor, Natural Factors) was added to 500 mL R/O water. The concentration of vitamin C was measured at 3 intervals over a 24-hour period. The half wafers were either added to the water whole or crushed with a mortar and pestle as in study 1. Containers with supplemented R/O water were added to the two exhibits housing GES, RACC/1 and RACC/6. The first exhibit (RACC/1) contained two vitamin C supplemented water containers, one with a half whole wafer and one with a crushed half wafer. The second exhibit (RACC/6) contained one vitamin C supplemented water container with a crushed half wafer. This was done to mimic the manner supplemented water was added by the keeper in the different exhibits. Containers were placed within the exhibit in areas not accessible by the GES.

Ascorbic acid measurements were conducted at initial placement of vitamin C in the water, at 4 hours, 6 hours, and 24 hours post supplementation. The temperature, light, and humidity were measured at each time period, as well. Water was collected (10ml) from each water container at each interval in each exhibit. Samples were wrapped in aluminum foil, frozen at -40 ^{o}F, and submitted on dry ice to Midwest Laboratory for determination of the amount of vitamin C that was present.

Ascorbic Acid Analysis

Samples for both Experiment 1 & 2 were submitted to Midwest Laboratories, Inc. Omaha, Nebraska. The level of ascorbic acid was determined according to the Midwest Laboratory protocol 10 based on AOAC 967.22. Samples were extracted with a weak acid solution. The extracts were derivatized with OPDA (o-Phenylenediamine) and analyzed with HPLC with a fluorescence detector at 430 nm emission.

Results

Table 1. Experiment 1: Concentration of Vitamin C (mg/100 g) in both tap and R/O water, at 0, 12, and 24 hours post supplementation.

	Concentration of Vitamin C (mg/100g)		
Water Type	**0 hours**	**12 hours**	**24 hours**
R/O H20	17.575	16.9375	16.5875
Tap H20	7.725	0	0

Table 2. Experiment 2: Concentration of Vitamin C in R/O drinking water (mg/100 g) from each GES exhibit as either whole half wafer or crushed half wafer at 0, 4, 6, and 24 hours post supplementation.

Collection Hour	**RACC 1 Whole mg/100 g**	**RACC 1 Crushed mg/100g**	**RACC 6 Crushed mg/100g**
0	0	23.8	15.3
4	18.4	21.2	18.6
6	33.3	24.5	22.7
24	15.9	24.7	14.9

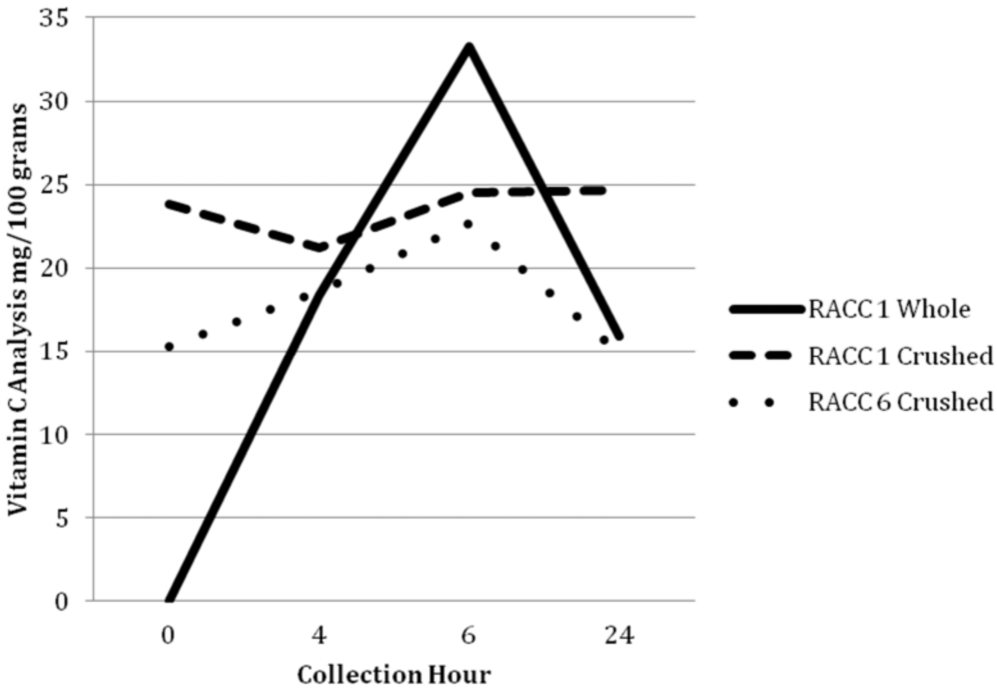

Figure 1. The concentration of vitamin C in each exhibit, either crushed half wafer or whole half wafer, over a 24 hour time period.

Discussion

The results of both experiments showed that supplementation of vitamin C (in wafers form) in R/O water is an appropriate means to provide vitamin C to mammals that require vitamin C in their diets.

The diet per adult giant elephant shrew at the Philadelphia zoo:
20 gms Eukanuba Adult Chicken Formula Cat Food
1 mL Giant Elephant Shrew Oil
10 gms Mealworms or 20 gms Crickets
5 kcal Keeper choice item
125 mg (1/2 wafer) Big Friends 100 % Natural Vitamin C added to 500 mL
R/O drinking water.

Eukanuba cat food is processed in the United States and is distributed by The Iams Company, a subsidiary of Procter and Gamble, Cincinnati, OH 45202. The feed is formulated to meet the nutritional levels established by the AAFCO nutrient profile for cats at maintenance. The feed does list ascorbic acid as a feed ingredient on the label; however, the nutrient level in the feed is not listed. Since, under normal conditions, cats are able to synthesize needed ascorbic acid from glucose in the liver and high levels of ascorbic acid intake is warned against by the NRC for cats (NRC, Nutrient Requirements of Dogs and Cats 2006), the amount of ascorbic acid added to the diet through this source is believed to be minimal. Repeated managerial intake studies conducted at the Philadelphia Zoo indicate the giant elephant shrews will consume between 8 -15 grams of the cat food daily (20 grams is considered an *ad libutum* allotment for this species). The nutrient contribution of 12 gms of cat food is used for diet formulation purposes. Giant Elephant Shrew oil is a combination of peanut oil, flaxseed oil and vitamin E liquid. The GES oil is added to the diet to increase the fat content of the diet as well as add omega-3 fatty acids and vitamin E. There is no ascorbic acid in the oil. Mealworm larvae have been reported to contain ~ 12 mg/kg Vitamin C and adult crickets 30 mg/kg as received from the commercial supplier on an as fed basis(Timberline Industries, Inc., Marion, IL) (Finke, 2002). Offered insects are routinely completely consumed by the GES. The keeper choice item can be any shrew appropriate feed item chosen by the keepers and can include fruit and vegetables as well as other insects. For the purpose of diet formulation, only the kcal added to the diet through keeper choice items are considered. The as-fed diet with no vitamin C supplementation only contains vitamin C from insects which is between 0.12 mg and 0.6 mg daily dependent on the insect chosen.

Once a pattern emerged in the deaths and subsequent necropsy reports of giant elephant shrews, suggesting that vitamin C may be necessary part of their diet, they were supplemented with vitamin C in their R/O drinking water.

Water intake studies conducted for managerial purposes indicate GES singles will drink an average of 39 – 61 mL of water daily and animals housed as pairs 70 – 90 mL daily. Supplementation of R/O water with ½ wafer (125mg vitamin C) Big Friends Tangy Orange Flavor 100% Natural Fruit Chews, manufactured by Natural Factors Canada and distributed by Natural Factors, 1111-80th St. SW, Ste 100, Everett WA, USA 98203 in both studies indicates the drinking water will maintain a minimum level of 14.9 mg/100 mL of water or .149 mg/mL water vitamin C over a 24 hour period. Based on the managerial water intake studies conducted at the Zoo the GES intake of vitamin C through supplemented R/O water ranges between 5.22 mg – 9.09 mg/day increasing the dietary intake of vitamin C to at least 5.34 mg/day or 178 – 236 mg/kg as fed diet. The estimated ascorbic acid requirement for growing guinea pigs is 200 mg/kg diet (NRC, 1995).

Anecdotally, there was a decrease in esophageal and/or gastric rupture in hand-reared neonates; a decrease in morbidity in hand-reared neonates due to pathologic vertebral fracture ascribed to collagen defects; a decrease in morbidity in adults due to periarticular disease. There was also an improvement in the overall health of the animals, which was noted on both opportunistic visual inspection and on routine physical examination.

Conclusion

The addition of crushed ascorbic acid (vitamin C) wafers to R/O drinking water is an appropriate and effective way to supplement vitamin C for animals that require vitamin C in their diet. Crushed half wafers added to the water maintain a more constant concentration over 24-hours than the whole have wafers which have to dissolve over time. Animals that choose to drink as soon as fresh water is added will not benefit from the vitamin C supplement. The addition of ascorbic acid to Philadelphia City tap water is of no value, as the vitamin completely loses activity within the first 6 hours.

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EVALUATION OF DIETS OFFERED TO ELEPHANTS IN BRAZILIAN ZOOS

Lucas Carneiro[1], Ana Raquel Faria [2], Gabriel Werneck[3], and Ellen S. Dierenfeld[4]*

[1]Zoológico de Pomerode, Rua Wemann Weege 180, Pomerode, Santa Catarina, Brasil 89.107-000
[2]Fundação Jardim Zoológico de Brasília, Av. das Nações, Via L4 Sul, Brasília, Distrito Federal, Brasil 70.610-100
[3]Universidade Federal do Paraná, Rua dos Funcionários, 1540, Juvevê, Curitiba, Paraná, Brasil 80035-050
[4]Ellen S. Dierenfeld, LLC, 4736 Gatesbury Drive, St. Louis, MO 63128

Abstract
In order to improve the quality of life for captive elephants maintained in Brazilian zoos, the Brazilian Society of Zoos and Aquariums (SZB) organized a workshop concerning management of elephants in zoos in November 2014. Evaluation of nutritional husbandry was identified as a priority. Thus, diets were assessed from information obtained for 4 African (*Loxodonta africana*) and 12 Asian (*Elephas maximus*) elephants held in 7 of 11 Brazilian zoos that maintain elephants. Animal body weights, diet ingredients and quantities offered were collected by survey; nutrient profiles of diets were assessed using local food composition tables and calculated using the software Zootrition®. Excess dietary energy (up to 280% of estimated needs) appeared most problematic, due to high amounts of foods offered, as well as elevated proportions of highly digestible components in the diets offered (up to 20% of calories from fruits and vegetables, and 33% from concentrate pellets in the dry matter (DM)). This may lead to overcondition (obesity) and concurrent health problems. A high level of sugar content (4.9 ± 2.5% of DM) was found in diets from four zoos that use sugarcane as forage; replacement with sugarcane bagasse (by-product of juice extraction) may be a way to decrease the sugar content and digestibility of those diets. A majority of the zoos surveyed (6/7) also fed diets that may have been higher in crude protein (10.9 ± 2.4% of DM) than necessary for maintenance requirements of adult elephants (~6 to 8% of DM, with values ranging from 7.6 to 15.4% of DM. As a result of the initial diet assessment, one zoo reformulated its diet to better meet calculated energy and protein needs;, a >50% reduction in dietary energy content, and slight increase in protein content, resulted in substantially improved body condition after 3 mo. Beyond identifying problems in the amount of energy supplied to elephants in Brazil, this study also shows that significant change is possible, provided that appropriate nutritional management is applied within the zoos.

Introduction
Classified by the IUCN red list (2014) as endangered and vulnerable, respectively, Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants are popular zoo exhibit species, and as such can provide excellent conservation and environmental education messaging. As with other species, appropriate nutrition and diets are critical for maintenance of healthy individuals. The natural diet of elephants has been extensively documented; free ranging elephants can be highly selective feeders or described as generalized herbivores, with diets varying according to habitat and seasonality (Dierenfeld, 1994, 2006). Spending 50 to 80% of the day feeding, both species are reported as consuming both monocots (grazers) and dicots (browsers), depending on the study, locale, and season; bark and fruits can also comprise a significant portion of native

diets. Nutritional summaries suggest that natural diet items contain moderate protein levels (typically, 8 to 13% of dry matter (DM), but ranging from 2 to 26%), high fiber concentrations (up to 82% NDF), and variable mineral content (Katole et al., 2014; Hatt and Clauss, 2006; Ullrey et al. 1997). Surveys of diets fed to elephants in US (Ange et al. 2001) and European (British and Irish Survey, 2001 unpubl; Nijboer and Casteleijn, 2002 unpubl) zoos, and two Thai captive facilities (Romain et al. 2014) documented 25 to 100 kg of dietary DM consumed per day (0.7-2.9% of body weights). DM digestibility coefficients range widely in both natural and captive diets – from approximately 30 to 80%, with grasses ~50% digestible, and legume forages, ~60% (Clauss et al., 2003; Das et al., 2015). To date, no evaluation of the diets offered to the elephants in Brazilian zoos has been published, thus, the zoo nutritionists can only utilize reference values established with animals maintained in different environmental conditions compared with those found in Brazil, and/or values based on extrapolations mainly with domestic horses, another large hindgut fermenter (Dierenfeld, 1994; Ullrey et al., 1997). Currently, 24 elephants are kept in Brazilian zoos, 7 African elephants (*Loxodonta sp*.) and 17 Asian (*Elephas maximus*), distributed across 11 zoos, (SZB, unpublished). The origin of those animals is varied, although most came from circuses, where they have been kept since a young age. Early diet history is unknown during this crucial stage of life, but we believe, based on verbal reports provided by old keepers, that diets consisted of: sugarcane (*Saccharum officinarum*), alfafa hay (*Medicago sativa*), mixed fruits, and commercial horse feed of unspecified ratios. This paper aims to summarize data from diets offered to elephants in Brazilian zoos, evaluate nutritional profiles, make recommendations for possible changes indicated, and aim towards standardized diet recommendations within the country's zoological community.

Materials and Methods

Data Collection

In November 2014, after a captive elephant management workshop attended by representatives of Brazilian zoos holding elephants, the need to evaluate diet quality was recognized. Each facility committed to send their diet record sheet for analysis to the Nutrition Working Group of the Brazilian Society of Zoos and Aquarium (SBZ). The nutritional composition of the items used in the diets was obtained from three national databases: the Brazilian Table of Food Composition TACO, 4th ed. (2011) was used for fruits and vegetables, the Brazilian Tables for Poultry and Swine, 3rd ed. (2011) was used for agricultural by-products, along with the online platform CQBAL 3.0 (2015), whereas label guaranteed analysis values were utilized for commercial foods; all data were entered into Zootrition® software (version 2.7, St. Louis, MO) for diet analysis.

Animals

Of 11 zoos that house elephants in Brazil, dietary information (ingredients and amounts offered) was obtained from 7 facilities, totaling 4 African elephants, 2 male and 2 female, and 12 Asian elephants, 1 male and 11 female (57% and 66% of the country's total individuals, respectively). All were adult animals, with ages ranging from ~20 to ~65 years. Actual or estimated body weights from individuals are found in Table 1. Of respondent zoos, only four provided actual animal weights; for the other individuals, estimated weights were based on photos and average species weight ranges (2500 – 3500 kg for Asian elephants and 4200 – 6000 kg for Africans). The daily energy requirements for adult maintenance (kcal/day) were estimated as [0.75 (143 kcal DE/BW $^{0.75}$)] from the equation suggested by Clauss et al. (2005) and used by Das et al. (2015), with a correction for reduced activity of the Brazilian elephants.

Results

Ingredients and Diets

Fruits and Vegetables: Thirteen different produce items were fed in varying quantities including cabbage, carrots, beets, pumpkin, sweet potato, corn on the cob, tomato, yucca, apples, bananas, oranges, papaya and watermelon. Amounts fed in the 7 facilities ranged from 0 to 58 kg/day/animal, averaging 27.7 ± 20.8 kg (fresh weights) or 4.8 ± 3.6 kg DM. Most popular items were bananas ranging from 0.1 to 2.3 kg DM (6 zoos), apples (0.1 – 0.8 kg DM) and papaya (0.1 – 1.8 kg DM) (5 zoos) and carrots (0.1 – 0.6 kg DM) (4 zoos).

Forages: Nine forages were utilized in the 7 zoos, including fresh elephant grass (*Pennisetum purpureum*; n=7 facilities), alfalfa hay (*Medicago sativa*; n=6), sugar cane (*Saccharum officinarum*; n=3), sugar cane leaves only (canes used for juice extraction) (n=2) and 1 facility each for fresh cut catalonha (*Cichorium intybus*), fresh corn stalks (*Zea mays*) or corn silage, fresh bristle oat grass (*Avena strigosa*), and Tifton 85 hay (*Cynodon* spp.). Total forage offered daily averaged 124.1 ± 42.0 kg, or approximately 41.3 ± 13.5 kg DM. Nutrient concentrations of forages used are found in Table 2.

Concentrates: Five different commercial equine products were used across 6 facilities; one fed no concentrate pellets. The average amount offered per elephant per day was 7.7 ± 5.2 kg (6.7 ± 4.5 kg DM). Nutrient concentrations of concentrates used are found in Table 3.

Diets for the various zoos comprised (average per animal, as-fed basis offered):

Zoo 1 – 78 kg forage (30 kg fresh sugar cane leaves, 24 kg alfalfa hay, 12 kg Tifton 85 hay, and 12 kg sugarcane), 58 kg mixed fruits and vegetables, and 4 kg equine pellets

Zoo 2 – 107 kg forage (90 kg fresh elephant grass, 17 kg alfalfa hay), 21 kg mixed vegetables and fruits, and 6 kg equine pellets

Zoo 3 – 205 kg forage (78 kg fresh elephant grass, 66 kg fresh sugar cane leaves, 48 kg fresh sugarcane, 13 kg alfalfa hay), 10 kg mixed vegetables and fruits, and 1.8 kg equine pellets

Zoo 4 – 100 kg forage (60 kg fresh elephant grass, 40 kg alfalfa hay), 12.8 kg mixed vegetables and fruits

Zoo 5 – 107.5 kg forage (100 kg fresh elephant grass, 5 kg alfalfa hay, 2.5 kg sugar cane), 50 kg mixed vegetables and fruits, and 10 kg equine pellets

Zoo 6 – 120 kg forage (72 kg elephant grass, 24 kg fresh bristle oat grass, 24 fresh corn plant), 14.5 kg mixed vegetables and fruits, 16.5 kg equine pellets

Zoo 7 – 151.2 kg forage (94.4 kg elephant grass, 22.8 sugar cane, 20 kg maize silage, 12 kg alfalfa hay, 2 kg catalonha), and 8 kg equine pellets

Amounts of food offered, both as-fed and on a dry matter basis, are displayed in Table 4. Dry matter offered ranged from 41.7 to 67.1 kg DM/d, averaging 51.3 ± 8.6 kg /d divided into 2 – 3 feeding times. Some zoos reported the use of equine trace mineral (TM) supplementation, but none provided the nutrient composition. Water was available ad libitum, and equine TM is used according to the manufacturer's recommendation for equines. No facility reported the use of plain salt.

Energy
The average amount of digestible energy (DE) offered to the animals was 98,824 ± 17,116 kcal DE/d considering the following digestibility coefficients:

Digestible Energy values were calculated for fruits and vegetables, and pelleted horse feeds, by multiplying Gross Energy (GE) by 0.8. For Forages, different digestibility factors were used: for catalonha, DE = 0.80 x GE; corn silage, DE = 0.40 x GE; alfalfa hay, DE = 0.46 x GE; sugar cane leaves, DE = 0.42 x GE; oat grass, DE = 0.55 x GE; ; corn plant, DE = 0.53x GE; Tifton hay, DE = 0.56 x GE; elephant grass, DE = 0.32 x GE;; sugar cane, DE = 0.54 x GE (DE values from CQBAL, 2015; Figueiredo, 1999; NRC, 2007). Estimated energy requirements, based on body weights can be found in Table 1, along with calculated calories (both Gross and Digestible) provided by the provisioned diets.

Energy concentration, fiber fractions, proximate composition, and Ca and P calculated in the diets are found in Table 5. Mean values of various fiber fractions calculated, on a DM basis, include: 27.6 ± 4.0 (% crude fiber), 33.1 ± 4.2 (% ADF), 48.8 ± 5.5 (% NDF), 23.1 ± 4.8 (% cellulose) and 5.8 ± 1.7 (% lignin). These values are more descriptive of grasses (monocots) compared with browses or dicots, yet fiber content appears to be low when compared to values that have been reported from forages consumed by free-ranging elephants (NDF levels up to 82% (Das et al., 2015). Of note, water soluble carbohydrates that could be quantified for some ingredients (and would generally be highly digestible) ranged from ~10 to 50% of DM, depending on the facility.

Protein
Most zoos fed diets with higher protein content (10.9 ± 2.4% of DM) than necessary for maintenance requirements of adult elephants (~6 to 8% of DM; Das et al., 2015; Ullrey et al. 1997), with values ranging from 7.6 to 15.4% of DM. These high values are attributed to the amount of concentrate fed (1.8 to 16.5 kg of fresh matter per individual per day), which provided ~4 to 53% of total dietary crude protein, as well as provision of the high-protein (18% CP) alfalfa hay (4.5 to 35.6 kg DM/d).

Minerals
Due to lack of information on mineral content of the commercial feeds, trace minerals in forages, and especially lack of data on any equine mineral supplements used, Ca and P were the only minerals evaluated in this initial diet analysis. The average calcium content of the diets (0.7 ± 0.2%) was higher than minimum dietary recommendations, whereas the average phosphorus content of the diets (0.2 ± 0.05%) was similar to suggested values for captive elephants (Ullrey et

al., 1997). However, the Ca:P ratio, was 3.2:1, higher than that recommended of 1.5:1, due primarily to the alfalfa component (21 to 90% of Ca supplied from the alfalfa).

Discussion

Most animals appeared to be overfed, both in quantity but also in some quality parameters (calorie, protein). Numerous studies document DM intake in elephants ranging from about 1.1 to 1.5, up to 1.9% of body weight (summarized in Dierenfeld, 2006; Hatt and Clauss, 2006; Ullrey et al., 1997). Animals in this survey were offered diets with DM amounts ranging from 0.8 to 2.7% of body weights (average 1.3 to 1.7%), thus the possibility of overconsumption and / or wastage of food was high for all facilities, with the exception of one institution (Zoo 2). More importantly, all zoos provided considerably more potential digestible energy for animals than needs estimated by prediction equations for maintenance (110% to 280%), with higher amounts provided to Asian elephants than Africans, in general. These high energy values offered can be attributed to excessive amounts of highly digestible ingredients (fruits and vegetables as well as concentrates), contributing 20% (fruits and vegetables) to as much as 33% (concentrates) of dietary DM calories. Further, these percentages may underestimate the contribution from highly digestible diet ingredients. As the fruit, vegetables, and concentrates are often highly preferred diet items, they can provide a disproportionate amount of total calories ingested by the elephants. Obesity is a documented health problem of captive elephants (Hatt and Class, 2006; Das et al., 2015) that can also negatively impact joint health and reproduction. All animals in this Brazilian survey were considered overconditioned (unpublished); an initial recommendation to address this issue, supported by this survey, was to reduce total amounts of food offered, but to particularly control or minimize the concentrate portion(s) fed. One Brazilian zoo feeds no concentrate, another feeds no fruits or vegetables, to elephants with no obvious negative outcomes. The zoo that fed no fruits or vegetables uses corn silage that contained a starch concentration of 22% DM; source of calories could also impact metabolism and body condition, as has been previously documented in elephants (Clauss et al., 2003; Das et al., 2015).

Zoos 1, 3, 5 and 7 reported sugarcane as part of the diet, although amounts of fresh cane fed varied radically from 2.5 to 48 kg per animal. Diets in these zoos analyzed with an average sugar content of 4.9 ± 2.5%, compared with an average 1.5 ± 0.4% sugar for those without sugarcane in elephants' diets. These high sugar levels (and highly available calories) may favor obesity, particularly in elephants that lead a sedentary life. Because sugarcane is common in Brazil, some zoos cultivate fields of the crop strictly for feeding their elephants, thus elimination or changing that crop to different forage may not be an immediate option. A practical alternative for these facilities may be to utilize sugarcane bagasse, a by-product from the sugarcane juice extraction, in the elephant feeding programs rather than the intact canes or whole plant. Comparing the two, sugarcane bagasse contains higher levels of fiber, 44.1 vs. 26.8% crude fiber, 85.7 vs. 54.3% NDF, and 59.0 vs 33.5% (all DM basis) than whole sugarcane, as well as considerably lower levels of water soluble carbohydrates (0.8 vs 35.0%). Thus processed cane bagasse may elevate dietary fiber levels, decrease digestibility, and is also much more economic than the sugarcane. We recommend that cane bagasse (estimated 27% digestibility) and/or leaves, rather than whole sugarcane fractions, be incorporated as forage sources in Brazilian zoo elephant diets. Elephant grass, at 32% digestibility, could also be used to replace some of the higher quality forages (i.e. sugarcane, Tifton and oat hays, alfalfa, and especially catalonha) to decrease dietary calorie concentrations for captive elephants.

Regarding dietary protein concentrations, the analyses reported may underestimate actual intakes, as the equine concentrate pellets (containing protein levels ranging from 12 to 17% DM) were consumed in entirety, while forages were not. Although the relatively high protein levels quantified here do not appear to cause problems for the adult animals at maintenance, and can certainly be necessary to meet needs for growth or reproduction (Das et al., 2015; Ullrey et al. 1997), they can be expensive to overfeed. If protein needs can be met by available forages (see Table 3) adjustment of diets to minimize pellets and meet the protein requirements with forage is suggested. Low protein forages (i.e. all sugar cane fractions, elephant grass and corn plants) can be blended with higher CP forages such as catalonha or alfalfa to meet protein needs.

The high Ca:P ratio found in the diet can be attributed to the high concentration of calcium found in alfalfa hay (1.3% DM), however there is no evidence that this increased amount of calcium, and consequent high Ca:P ratio is harmful to elephants. Clearly more detail is needed for other minerals in the diets of these animals for optimal health and nutritional assessment, and is recommended for future action.

Soon after the initial diet analysis, zoos were provided reports with suggestions to improve nutritional balance and minimize waste. Zoo 3 had 2 animals with clear signs of obesity (body condition scores 8 and 7 in a 1-9 scale (Wemmer et al., 2006)). They offered to immediately make changes in their diets in order to improve the quality of life of the animals. Following implementation of proposed changes in the diet (Tables 6-7), the DM intake was reduced from 2.2 to 0.7 %BW (animal 1) and from 2.7 to 0.8 %BW (animal 2). Energy amounts were reduced to approximately 1/3 the original levels, from ~122,000 kcal to ~37,000 kcal/day for animals weighing between 2500 to 3000 kg (with an estimated energy requirement of ~38,000 to ~43,000 kcal/day), corresponding to approximately 90-100% of estimated energy needs., and crude protein concentration was increased from 7.6 to 9% DM. Also, a conditioning program was implemented with daily training sections. After 3 months, an obvious reduction in body condition scores can be seen in Figures 1 and 2. The results obtained by zoo 3 are considered satisfactory, and they can be regarded as a reference so that other elephants in the same obesity conditions may gradually improve body condition. As with all herbivores, diet changes should be made slowly (over weeks to months) and, in the case of dramatic reduction in quantities, it is essential that the nutritional profile of the total diet be assessed to ensure that known or estimated nutritional needs are being met.

Initial Recommendations for Brazilian Zoo Elephant Diets

1. Maintenance diets should be formulated to provide 1-1.5% of body weight as dry matter intake. Lower quantities, or poorer quality ingredients, are required for weight loss diets.
2. Energy; pelleted concentrate feeds and fruits and vegetable amounts can be minimized and carefully controlled to provide no more than ~10% of calculated calorie needs and optimize nutrient balance as well as feeding economics.
3. Nutrient target concentrations should be developed as per recommended by the AZA Nutrition Advisory Group, using equine requirements as primary guidelines.
4. The use of whole sugar cane in feeding programs, with its high calorie content from readily available sugars, is discouraged. Rather, cane bagasse, following juice extraction, can be offered to elephants as a fiber source, in addition to other forages to balance

nutrients. Elephant grass is particularly suggested as a suitable and economic forage, but nutrient quality must be monitored and maintained.

5. More detailed analyses of mineral content of commercial equine feeds, forages, and especially mineral supplements fed to elephants in Brazilian zoos is needed to better analyze, balance, and optimize feeding programs for these species.

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Table 1. Estimated daily maintenance energy requirements (MER, kcal) of elephants in 7 Brazilian zoological facilities, with calculated Gross Energy (GE) and Digestible Energy (DE) values provided by diets.

	Unit	Zoo 1	Zoo 2	Zoo 3	Zoo 4	Zoo 5	Zoo 6	Zoo 7
Weight Animal 1	kg	3.000	6.000	3.000	3.500	3.000	3.000	3.800
Maint Energy Reqt	Kcal DE/d	43.475	73.116	43.475	48.803	43.475	43.475	51.908
Calc Dietary Energy	Kcal GE/d	220.892	187.250	279.585	207.704	163.060	194.765	204.750
Calc Dietary Energy	Kcal DE/d	117.113	90.088	122.093	89.249	80.628	107.475	91.046
Weight Animal 2	kg	3.000	6.000	2.500	2.600	2.500		3.800
Maint Energy Reqt	Kcal DE/d	43.475	73.116	37.919	39.051	37.919		51.908
Calc Dietary Energy	Kcal GE/d	220.892	187.250	279.585	207.704	163.060		204.750
Calc Dietary Energy	Kcal DE/d	117.113	90.088	122.093	89.249	80.628		91.046
Weight Animal 3	kg	3.000	5.000					4.200
Maint Energy Reqt	Kcal DE/d	43.475	63.771					55.954
Calc Dietary Energy	Kcal GE/d	165.669	146.726					219.927
Calc Dietary Energy	Kcal DE/d	87.835	70.592					97.794

Table 2. Nutritional composition of forages fed to elephants in Brazilian Zoos.

	Unit	Alfalfa Hay	*Avena strigosa*	Sugar cane	Elephant Grass	Catalonha	Corn stalks	Sugar cane leaves only	Corn silage	Tifton Hay
Dry Matter	%	89	17	28	21	9	33	35	31	89
Gross Energy	kcal/g	4.22	4.10	4.13	4.12	2.67	4.27	4.29	4.33	4.29
						Carbohydrates				
ADF	%	37.52	31.00	33.52	46.24		28.05	41.31	30.67	39.32
Cellulose	%	29.42		28.37	38.00		25.92		26.91	32.03
Crude Fiber	%	29.36	31.50	26.79	35.75		25.02	43.70	24.90	32.34
Lignin	%	9.74	4.50	5.86	7.85		4.66		4.87	5.13
NDF	%	46.93	54.30	54.29	76.00		55.25	54.64	54.52	77.68
Starch	%	4.64		4.15			6.95		22.56	1.20
Sugar	%			19.00						
Water Soluble Carbohydrates	%	69.11		35.00		53.33				6.57
						Fat And Protein				
Crude Fat	%	2.85		1.34	2.20	3.33	2.06	2.24	2.84	1.75
Crude Protein	%	18.77	11.60	2.76	6.80	21.11	7.06	4.93	7.24	9.12
						Ash/minerals				
Ash	%	9.11	3.45	3.12	9.02	14.44	4.51	6.23	5.07	7.10
Calcium	%	1.30	0.34	0.23	0.34	0.63	0.16	0.31	0.31	0.50
Phosphorus	%	0.24	0.20	0.08	0.23	0.36	0.08	0.06	0.19	0.18
Ca:P	Ca/P	5.42	1.70	2.88	1.48	1.78	2.00	5.17	1.63	2.78

Table 3. Nutritional composition of concentrates fed to elephants in Brazilian Zoos.

	Unit	CRAVIL EQUINOS MANUTENÇÃO	GUABI NUTRIAGE 15 LAMINADOS	NUTRINA EQUINOS	PRESENCE	SUPRA EQUINOS
Dry Matter	%	88	87	87	87	88
Gross Energy	kcal/g	3.25	3.40	2.35	3.81	3.39
ADF	%		10.00		11.49	
Crude Fiber	%	12.00	12.00	10.00	11.49	16.00
Crude Fat	%	3.50	4.00	2.00	4.02	2.00
Crude Protein	%	12.50	15.00	17.00	17.24	12.00
Ash	%	9.90	12.00	10.00	14.94	15.00
Calcium	%	1.20	1.50	1.50	1.38	2.00
Copper	mg/kg	12.00	3.45			
Phosphorus	%	0.60	0.50	0.60	0.57	0.40
Ca:P	Ca/P	2.00	3.00	2.50	2.40	5.00

Table 4. Total amounts of diet (as fed and dry matter) offered to elephants in Brazilian zoos, dry matter (DM) offered as a % of body weight.

	Unit	Zoo 1	Zoo 2	Zoo 3	Zoo 4	Zoo 5	Zoo 6	Zoo 7	Average	SD
Total	kg	140	134	216.8	112.8	167.5	151	159.2	154.5	32.8
Dry Matter	%	40.8	36.6	31	44.2	25.2	28.9	31.6	0.3	
Dry Matter	kg	57.1	49.0	67.2	49.9	42.2	43.7	50.3	51.3	8.5
Animal 1	DM%BW	1.9	0.8	2.2	1.4	1.4	1.5	1.3	1.5	
Animal 2	DM%BW	1.9	0.8	2.7	1.9	1.7	-	1.3	1.7	
Animal 3	DM%BW	1.9	0.8	-	-	-	-	1.2	1.3	

Table 5. Calculated composition of diets (select nutrients) fed to elephants in Brazilian Zoos. All values (except dry matter, DM) on a DM basis.

	Unit	Zoo 1	Zoo 2	Zoo 3	Zoo 4	Zoo 5	Zoo 6	Zoo 7	Average	SD
Gross Energy	kcal/g	3.9	3.8	4.2	4.2	3.9	4.5	4.1	4.1	0.2
Digestible energy	% GE	53.0%	48.1%	43.7%	43%	49.4%	55.2%	44.5%	48	
Carbohydrates										
ADF	%	31	31.3	38.77	38.45	29.98	27.77	34.22	33.1	4.24
Cellulose	%	18.7	23.72	20.07	30.59	22.78	17.86	28.14	23.1	4.80
Crude Fiber	%	27.71	23.90	34.58	30.00	24.49	23.69	29.03	27.6	3.98
Lignin	%	4.95	6.42	4.78	8.93	5.10	3.98	6.50	5.8	1.65
NDF	%	45.34	47.91	56.44	52.68	44.25	41.40	53.52	48.8	5.54
Starch	%	3.11	2.26	1.68	3.70	2.63	1.70	4.29	2.8	0.99
Sugar	%	4.62	1.87	4.04	1.17	8.37	1.40	2.41	3.4	2.55
Total Dietary Fiber	%	1.85	0.60	0.23	0.54	1.87	0.61	0.08	0.8	0.74
Water Soluble Carbohydrates	%	33.80	36.20	12.71	50.18	9.70	2.12	14.86	22.8	17.39
Fat And Protein										
Crude Fat	%	2.14	2.28	2.18	2.65	2.37	2.48	2.28	2.3	0.18
Crude Protein	%	11.68	11.43	7.61	15.38	9.60	10.75	9.66	10.9	2.42
Ash/minerals										
Ash	%	7.58	7.73	6.87	8.93	8.60	9.37	8.66	8.2	0.88
Calcium	%	0.79	0.70	0.49	1.02	0.64	0.64	0.76	0.7	0.16
Copper	mg/kg	0.70	0.20	0.40	0.11	1.62	0.17	0.11	0.5	0.55
Iron	mg/kg	2.66	1.07	49.99	0.74	4.56	0.85	1.23	8.7	18.25
Manganese	mg/kg	2.45	0.70	29.71	0.31	3.46	0.91	0.14	5.4	10.79
Phosphorus	%	0.18	0.26	0.15	0.23	0.26	0.31	0.23	0.2	0.05
Potassium	%	0.22	0.98	0.61	0.65	1.38	0.89	0.95	0.8	0.36
Zinc	mg/kg	1.79	0.46	49.89	0.44	3.24	0.53	0.20	8.1	18.47
Ca:P	Ca/P	4.32	2.74	3.24	4.35	2.43	2.07	3.27	3.2	0.88

Table 6. Estimated daily maintenance energy requirements (MER, kcal) and dry matter intakes (DMI, %BW) of elephants in Zoo 3, before and after diet changes, with calculated Gross Energy (GE) and Digestible Energy (DE) values provided by diets.

	Unit	Before diet change	After diet change
Weight Animal 1	kg	3000	3000
DMI	%BW	2.2	0.7
Maint Energy Reqt	kcalDE/d	43,475	43,475
Calc Dietary Energy	kcalGE/d	279,585	83,400
Calc Dietary Energy	kcalDE/d	122,093	37,682
Weight Animal 2	kg	2500	2500
DMI	%BW	2.7	0.8
Maint Energy Reqt	kcalDE/d	37,919	37,919
Calc Dietary Energy	kcalGE/d	279,585	83,400
Calc Dietary Energy	kcalDE/d	122,093	37,682

Table 7. Zoo 3 elephant diet amounts and ingredients before and after suggested changes.

	Before diet change			**After diet change**		
	DM (g)	As Fed (g)	% DM	DM (g)	As Fed (g)	% DM
CENOURA/CARROT	200	2,000	0.30%	800.00	8,000,00	3.9%
ABOBORA/PUMPKIN	240	2,000	0.36%	960	8,000	4.6%
MAÇA FUJI, COM CASCA/APPLE IN SHELL	80	500	0.12%	160	1,000	0.8%
TOMATE/TOMATO	100	2,000	0.15%	0	0	0.0%
MAMÃO/PAPAYA	120	1,000	0.18%	0	0	0.0%
BANANA	115	500	0.17%	230	1,000	1.1%
LARANJA/ORANGE	65	500	0.10%	0	0	0.0%
MILHO ESPIGA/CORN COB	0	0	0.00%	870	1,000	4.2%
MELANCIA/WATERMELON	150	1,500	0.22%	100	1,000	0.5%
ALFAFA,FENO/ALFAFA HAY	11,570	13,000	17.23%	3,560	4,000	17.1%
PONTA DE CANA/SUGAR CANE AERIAL PART	23,100	66,000	34.40%	7,000	20,000	33.7%
CAPIM ELEFANTE/ELEPHANT GRASS	16,380	78,000	24.40%	6,300	30,000	30.3%
CANA/SUGAR CANE	13,440	48,000	20.02%	0	0	0.0%
CRAVIL EQUINOS MANUTENÇÃO	1,584	1,800	2.36%	780	1,000	3.8%
Total (g)	67,144	216,800		20,760	75,000	

Figure 1. Body score estimative before diet change (1) and after (2) to animal 1 from zoo 3.

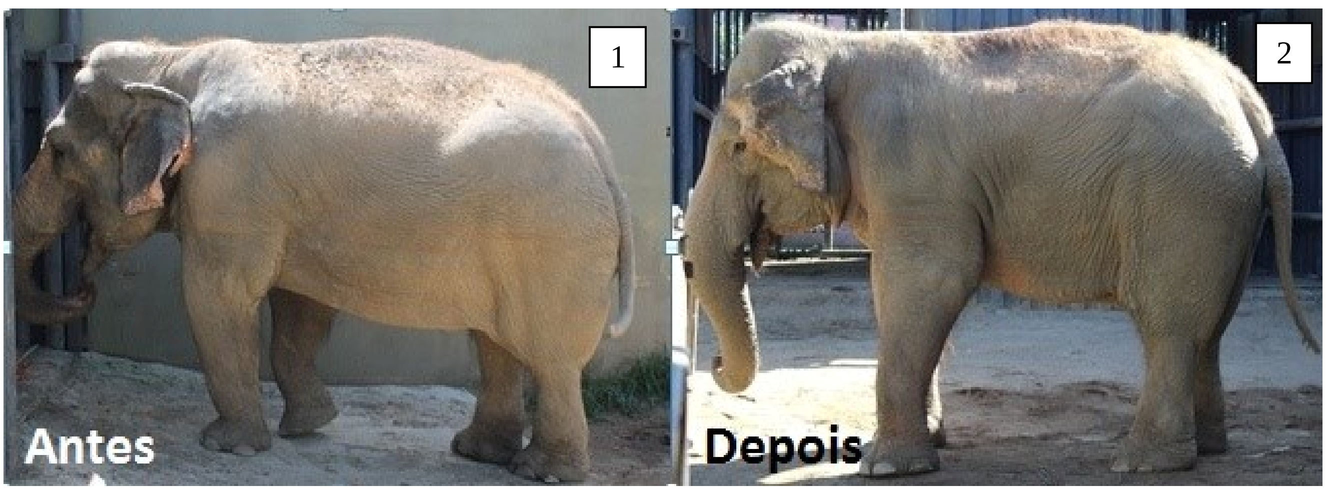

Figure 2. Body score estimative before diet change (1) and after (2) to animal 2 from zoo 3.

USE OF A NOVEL IRON CHELATOR (HBED) IN BLACK RHINOCEROS

Kathleen E. Sullivan[1,3]*, Eduardo V. Valdes[1-5], Shannon E. Livingston[1], Mitchell D. Knutson[3], Charles R. Staples[2], Lori K. Warren[2], Shana R. Lavin[1,2]

[1]Department of Animal Health, Disney's Animal Kingdom, Lake Buena Vista, FL 32830, USA
[2]Department of Animal Sciences, University of Florida, Gainesville, FL 32611, USA
[3]Food Science & Human Nutrition Department, University of Florida, Gainesville, FL 32611, USA
[4]University of Guelph, Ontario, Canada.
[5]University of Central Florida, Orlando, FL 32816 USA.

Abstract

Black rhinoceroses (*Diceros bicornis)* are critically endangered and are compromised by iron overload under human care (Paglia and Tsu, 2012; Molenaar et al., 2008). With the goal of safely reducing iron absorption in the gastrointestinal tract of affected and susceptible individuals, we investigated oral administration of the iron chelator, N,N-bis(2-hydroxybenzyl)ethylenediamine-N,N–diacetic acid (HBED) to 2.1 black rhinoceros. We expected HBED administration with diet to increase iron excretion, without compromising the health of the black rhino as assessed by blood parameters. Previously, HBED was tested thoroughly for toxicity and iron elimination efficacy in rats, non-human primates, dogs and through phase 1 clinical studies in humans (Bergeron, 1999; Grady and Hersko, 1990; Grady, 1994). Furthermore, our study was completed after our successful safety and efficacy study in the horse (Sullivan et al., 2014), the most appropriate digestive model for black rhinoceroses (Clauss et al., 2007). In the black rhinoceros study, we used a crossover design to compare iron excretion with and without HBED (40 mg/kg BW/day). Rhinos consumed 100% of HBED or control dosed with the diet. Rhinos excreted significantly more iron in the urine when administered HBED (349 ± 57 ng/ml) versus control (78 ± 13 ng/ml; $P<0.05$). Fecal iron excretion was not significantly different between treatments. The males did not show changes in serum parameters, but were not considered highly overloaded during the study. While HBED successfully sequestered and excreted iron via the urine and appeared to immediately decrease serum iron saturation in the iron-overloaded female, medical complications in the female animal's case raise concern about the safety of HBED administration in iron-overloaded individuals (Sullivan et al., 2015). Although demonstrated efficacy of this chelation treatment could potentially prevent or manage iron overload in black rhinos under human care, extra caution should be taken before further testing is initiated in compromised and/or iron-overloaded individuals. Iron overload disorder threatens the health and survival of black rhinos managed under human care, and research must be continued to find the safest effective methodology.

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VITAMIN E SUPPLEMENTATION IN AFRICAN ELEPHANTS

Katherine Kerr[1,2]*, Kathleen Sullivan[1,2], Shannon Livingston[1], Charles Staples[2], Eduardo V. Valdes[1-4]

[1]Disney's Animal Kingdom, Lake Buena Vista, FL 32830 USA.
[2]University of Florida, Gainesville, FL 32611 USA .
[3]University of Guelph, Ontario, Canada.
[4]University of Central Florida, Orlando, FL 32816 USA.

Abstract

Vitamin E, an essential vitamin, has antioxidant properties that are important for maintaining optimal health. In the 1990's, comparisons of zoo-housed and wild elephant species revealed concentrations of blood a-tocopherol (vitamin E) in zoo-housed elephants of approximately half that seen in their wild conspecifics (0.4 vs. 0.8 ug/ml; Dierenfeld and Traber, 1992). Addition of dietary vitamin E supplement for the adult African elephants housed at Disney's Animal Kingdom (DAK) was initiated in 2003 (2 to 6 IU/kg; water soluble a-tocopherol). We utilized longitudinal (1999 to 2014) serum vitamin E paired with age data (0 to 3.36 ug vitamin E/ml; 1.8 to 37.7 y old) from 18 elephants (*n* = 12 female; *n* = 6 males) housed at DAK to evaluate changes in serum vitamin E concentrations with age (elephants with no dietary supplementation), and the impacts of dietary vitamin E supplementation on serum values.

Decreases in serum vitamin E concentrations with age: A subset of data (*n* = 159 observations) were chosen for which no dietary vitamin E supplementation was reported in historical diet records. Serum vitamin E correlated negatively with age for 2 to 6 y-old elephants (R^2 = -0.51; P < 0.001), but not for 6 to >10 y-old animals (R^2 = -0.089; P = 0.329; PROC CORR, SAS). Serum vitamin E was highest (P < 0.05) in 2 y-old animals (1.5 vs. 0.4 to 1.0 ug/ml in animals 3 to >10 y-old), while serum vitamin E was lowest (P < 0.05) in 8 and >10 y-old animals (0.4 to 0.5 vs. 0.8 to 1.5 ug/mL in animals 2 to 4 y-old; PROC MIXED, SAS).

Dietary vitamin E supplementation & serum concentrations: Utilizing all the data (n = 348 observations), we determined that younger elephants (7 y-old and younger; no supplementation) had higher (*P* < 0.01) mean serum vitamin E (0.86 ± 0.14 ug/mL) compared to older elephants. Within older elephants (> 7 y-old), mean serum vitamin E was higher (*P* < 0.01) in elephants fed supplemented diets (0.70 ± 0.13 ug/mL; 2 to 5 IU/kg BW) versus those fed diets without additional supplementation of vitamin E (0.41 ± 0.13 ug/mL; 0 IU/kg BW; PROC MIXED, SAS). Mean serum vitamin E was not different (*P* > 0.05) than 0.8 ug/mL (*i.e.*, the value reported for wild elephants) for younger animals and older supplemented animals; while serum vitamin E of un-supplemented older animals was lower (*P* < 0.01) than 0.8 ug/mL (T-test, SAS).
Dietary vitamin E supplementation has the potential to increase serum concentrations of zoo-housed African elephants to concentrations similar to those reported for their wild conspecifics; however, the physiological benefit of this has not yet been elucidated. These data indicate that the appropriate time to begin considering dietary supplementation of vitamin E for captive elephants is approximately 6 to 8 y-old. Necessity of supplementation should be determined by evaluating blood serum. Due to the high variability of serum vitamin E, low serum

concentrations should be confirmed. Post supplementation serum E should be evaluated to determine efficacy of dosage.

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CASE STUDY: A HEMOLYTIC EVENT IN AN IRON OVERLOADED BLACK RHINOCEROS (*DICEROS BICORNIS*) IN ASSOCIATION WITH CESSATION OF CHELATION THERAPY

Kathleen E. Sullivan, MS[1]*, Natalie D. Mylniczenko MS, DVM, Dipl ACZM[1], Jessica A. Emerson DVM[1], Natalie H. Hall DVM, Dipl ACZM[1], Deidre Fontenot DVM[1], Ryan De Voe DVM, MSpVM, Dipl ACZM, Dipl ABVP[1], Elizabeth Nolan DVM, Dipl ACZM[1], Nicole Stacy DVM, DrMedVet, DACVP[2], Shannon E. Livingston MSc[1], Shana R. Lavin PhD[1], Eduardo V. Valdes PhD[1], and Geoffrey W. Pye BVSc, MSc, Dipl ACZM[1]

[1]Animals, Science, and the Environment, Disney's Animal Kingdom, Orlando, FL 32830, USA
[2]Department of Large Animal Clinical Sciences, University of Florida, College of Veterinary Medicine, Gainesville, FL 32608, USA

CREATION OF THE ZOO AND WILDLIFE NUTRITION FOUNDATION

Barbara A. Henry[1]*, MS, Michael T. Maslanka[2], MS, Ann M. Ward[3], MS, Shannon Livingston[4], MSc, Howard Fredrick[5], Ph.D, PAS, Kerri A. Slifka[6], MS, Barbara Toddes[7], BS, PGC

[1]Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220, USA
[2]Smithsonian Conservation Biology Institute, National Zoological Park, Center for Animal Care Sciences, Department of Nutrition Science, 3001 Connecticut Ave., Washington, DC 20008, USA
[3]Fort Worth Zoo, 1989 Colonial Parkway, Fort Worth, TX 76110, USA
[4]Disney's Animal Kingdom, PO Box 1000, Lake Buena Vista, FL 32830, USA
[5]PO Box 1890, Cortao, AZ 85652, USA
[6] Dallas Zoo, 650 S. R.L. Thornton Fwy, Dallas, TX 75203, USA
[7]Philadelphia Zoo, 3400 West Girard, Ave, Philadelphia, PA 19104 USA

Introduction to the Zoo and Wildlife Nutrition Foundation (ZWNF)

The Zoo and Wildlife Nutrition Foundation (ZWNF) was formed in March 2012 and became an official 501(c)3 organization a year later. The Foundation was established for the purpose of contributing expertise and providing funds to support the field of zoo and wildlife nutrition worldwide, furthering the science of zoo nutrition, facilitating the dissemination of nutrition knowledge, and developing the next generation of zoo nutrition leaders. To that end, a memorandum of understanding (MOU) was enacted in 2015 that defines the role of the Foundation in its support of the Association of Zoos and Aquariums (AZA) as a whole and, specifically, the goals and mission of the AZA Nutrition Advisory Group (NAG). One of the primary goals of the ZWNF is to provide support and fund-raising capacity for the Susan Crissey Animal Nutrition Residency Fund, a fund to train future zoo nutritionists. Additionally, the Foundation provides technical, financial and administrative support for the NAG website. In these ways, the ZWNF functions in concert with the AZA NAG to support the effective and efficient accomplishment of common goals to benefit the zoo nutrition profession.

Governance of ZWNF

The Board of Directors provides direction and management of the Foundation. The Board is currently comprised of 7 dues paying members from 7 different facilities around the US. They are elected by an approved set of bylaws to serve 3 year terms, and then rotate from the Board to ensure new ideas and perspectives as the Foundation grows over time. Initially, these individuals comprised a group of interested professional zoo nutritionists with a vested interest in the success and future growth of the NAG and its goals. Over time, the focus of the Board likely will change as fund-raising will be pivotal to the future collective success of the Foundation and the NAG.

Specific Project/Activity Goals of the ZWNF

The ZWNF provides support (primarily financial) to the mutual nutrition related goals of both AZA and the NAG. This includes but is not limited to: the SCARF program, the continued development and maintenance of an engaging educational website, and the ongoing efforts to conduct an efficient and effective biennial conference on zoo and wildlife nutrition. All of these efforts revolve around the financial ability of the Foundation to support those needs. As a tax exempt nonprofit, the ZWNF is freed from taxes and potential donors can make tax-deductible contributions. These benefits are critical to a fund raising organization. Thus, over time, the goals of the Foundation increasingly will include fund-raising. The financial resources provided by

ZWNF, coupled with the operational and logistical resources of the NAG, will allow for the timely achievement of goals, and the further development of the organizations and the field as a whole. As part of the MOU established among the three partners (AZA, NAG, ZWNF), there also remains the possibility of the Foundation providing grant support to specific NAG-approved research projects that are consistent with the mission and priorities of the ZWNF, in a similar fashion to other Foundations established in the same spirit (IRF, IEF, etc). This aspect will take some time to develop.

Ultimately, the goal of the Founding Board of Directors of the ZWNF will be to provide an additional resource for the benefit of the profession as a whole. Initially, the goals of the Foundation are obvious, based on the current needs of the NAG. As those needs change, the Foundation will be well poised to continue its support of NAG goals into the future, contributing to the collective success of both organizations and the field.

FINANCIAL ASPECTS OF ZOO DIET MANAGEMENT

*Mike Maslanka, MS**

Smithsonian Conservation Biology Institute, National Zoological Park, Center for Animal Care Sciences, Department of Nutrition Science, 3001 Connecticut Ave., Washington, DC 20008, USA

Introduction

Food purchases tend to be one of the greatest expenses, beyond salaries and benefits, for the maintenance of any zoo collection. For this reason, the commissary/food budget is one that receives a lot of attention during the budget planning process. Upon each accreditation inspection, the Association of Zoos and Aquariums (AZA) requires proof of financial stability, including insurance coverage, evidence of financial support that outpaces expenses, and a backup plan in case funding is reduced. However, reviews are made once every five years and details of the commissary portions of the zoo's budget and their purchasing practices are seldom reviewed in detail. Because of this, it is important that each zoo routinely and regularly scrutinize the animal food budget, purchasing practices, and overall diet management/formulation processes to ensure the most prudent use of resources – financial and otherwise.

Diet formulation/Ingredient choices

Appropriate diets can be formulated using a wide variety of ingredients, not necessarily the most or least expensive. For each nutritional need, a variety of ingredient options exist to meet that need, and the selection of one option over another is often a cost-benefit analysis or a risk assessment. The costs (or cost savings) are not necessarily always evident upon first evaluation, and care should be taken to ensure choices meet not only the financial concerns, but more importantly the animal care needs of the animals in question. Remember, "purchasing poor quality food at any price is not only poor nutritional management, but bad economics as well" (Watkins, 1984).

Diet items that are not consumed by the target animals are wasted, in terms of the physical item itself, the nutrients it contains, and the money used to purchase it. There may be a variety of reasons for a food item to not be consumed: poor initial quality, degradation in storage, overfeeding, and/or lack of preference, among others. Avoiding waste, regardless of the cause, is imperative for a financially efficient operation.

The use of nutritionally complete feeds (biscuits, pellets, etc) is often viewed as more expensive than using other items. While this may sometimes be the case, it is often not accurate. On a dry matter basis (water removed from the ingredients), nutritionally complete feeds are often much more cost effective than produce or other items, which may appear comparable on the face of things (Table 1). While nutritionally complete feeds do represent a financial investment, many are formulated to meet specific needs of the animals in question and, with appropriate consumption, can save time and effort in avoiding clinical or sub-clinical metabolic disorders (a cost-savings that is not especially evident or obvious). Evaluation of ingredients and their ability to help meet nutrient needs of the collection is invaluable. Sometimes expensive ingredients have been maintained on inventory for no practical reason aside from history and, upon evaluation, can be replaced or removed without concern (or even to the benefit of the overall diet).

Table 1. Comparative cost of common nutritionally complete food items and produce (aka – "water is not cheap").

Ingredient	Cost per Pound, as fed	Cost per Pound, dry matter basis (DMB)
Primate biscuit	$1.40	$1.47
ADF-25 pellet	$0.49	$0.51
Papaya	$2.73	$22.75
Grapes	$1.76	$8.80

The use of donated food items or "seconds" can potentially be a cost-effective way to provide diets for collection animals. There are a variety of concerns with this practice in order to ensure the apparent cost savings are real. These concerns focus on evaluating the quality and cost (apparent and hidden), of the ingredients and practices. It is imperative that food items, regardless of source, are evaluated for quality. Items that have passed their expiration date, have detectable signs of degradation, or appear abused should be viewed with caution and likely not included in zoo animal diets (unknown nutrient content, unknown changes associated with degradation/decay, etc.; Henry et al., 2010). In cases where donated produce is considered for use, the increased labor investment in procurement, a quality sort, and trimming may offset the perceived savings in the initial donation. In addition, if the donated items are not ones routinely used in diets, nor can be predictably available over time, the increased labor of determining nutritionally-appropriate substitutions may offset the perceived savings.

Multiple sources of meat and meat mixes are available for use in zoos, across a wide range of cost and quality. Care should be taken to evaluate the quality of the meat or meat product to ensure that it meets the nutrient and sanitation standards necessary for your carnivore collection, and not rely solely on the financial assessment. Again, just because an item is "free" or apparently more cost-effective than another, does not mean that "hidden expenses" do not exist (compromised health and welfare, increased labor, etc). The AZA Nutrition Advisory Group has a cautionary statement regarding the use of roadkill in zoo animal feeding programs (http://nagonline.net/guidelines-aza-institutions/nag-carcass-feeding-statement/). Regardless of cost, the use of 3D and 4D meat as a cost-savings measure is not recommended beyond the cautionary statements in the above reference (Crissey et al., 2001). When using larger carcass pieces, it is important to evaluate the product not only in terms of total per pound cost (meat mixes will not include bone weight, but carcass purchase may), but also in terms of total value (which is sometimes abstract) to the collection animal in question. It is often difficult to compare the benefits of carnivore diet ingredients solely on price, when additional animal care considerations (behavioral and/or physiological benefits) exist.

Purchasing Practices and Inventory Management

Not every zoo animal needs to have an individual diet or set of diet ingredients in order to meet their nutritional needs. Diets are expected to meet the nutritional needs of the animal in question (period). This offers some hope to the commissary manager trying to keep their total ingredient inventory reasonable (i.e. not a lot of single use items), at the same time allowing bulk purchase of some feeds in order to save money. Fish, meat, nutritionally complete feeds, and hay, based on available storage space, can be purchased in bulk to reduce costs associated with shipping smaller loads, or paying the vendor for longer storage times elsewhere. In addition, bulk purchase can allow for better nutritional management (need to sample fewer lots of product), as long as recommended storage times or expiration dates are not exceeded. Care must be taken, however, to ensure that the amounts ordered can be used in a timely fashion, or else the cost savings of a bulk purchase will be lost when the unused or degraded product is discarded. In the case of fish purchases, the biology of the fish species should be considered (i.e. runs and is caught once per year vs. multiple times per year). If the fish species is a once per year catch, timing the purchase for as close to the catch date as possible ensures the freshest product available at the lowest price (once the supply dwindles and/or the product has been in storage longer, the price increases over time). However, based on the prevailing purchasing philosophy of the zoo, and proximity to vendors that provide each individual food item, it may be more cost effective to have less on-hand inventory (money tied up in stored product), sparing funds for other purchases or aspects of the operation.

In certain situations, vendors will allow (or even encourage) a contract to be established for a pre-defined period of time. "Knowing" that the supply of the feed in question has been "secured" can be a benefit in certain situations, but requires an evaluation of the price compared to purchasing off contract. In some situations, the benefit of the guaranteed business may allow for a reduced price from the vendor. In other situations, because the vendor has to account for the possibility of a price increase during the term of the contract, the price may be inflated compared to the current market, to protect the vendor from the uncertainty of the future.

In all cases, whether single purchases, long term historic purchases, or contact purchases, routine price comparisons among qualified vendors are advised. This ensures that any changes in market prices are detected and acted upon to the benefit of the zoo (when possible). In addition, ongoing evaluation of each vendor is advised. Poor customer service (in broad terms – not just returning calls and inquiries, or providing poor quality items upon delivery, but extended/unpredictable periods between order and delivery of product, lack of understanding the mission at hand, etc) can easily increase the cost of otherwise affordably priced items (in terms of labor, communication, follow up).

The decision to grow or raise food items "in house" often appears prudent until labor is considered. Whether growing and harvesting hay/browse, or maintaining a feeder insect, bird, or mammal colony, the costs and benefits should be evaluated continuously. Having supplies of food items that are maintained on zoo grounds can be a benefit to those charged with maintain those supplies, as well as those using them through the facility (potentially minimizes supply chain issues and may or may not reduce cost). Often, the labor invested in maintaining these in house production systems is not considered as part of the "cost." Labor, in terms of actual real time spent, and in terms of opportunity cost (other tasks not completed during the same time), is not free. In many cases, the infrastructure, equipment, and labor costs easily outstrip the perceived cost savings compared to purchasing the same feeds rather than producing them in house, but this should be critically evaluated prior to initiation and throughout use.

Diet Preparation Plan

The choice of a centralized or decentralized food operation also can have financial ramifications, and the two systems are well detailed in a previous paper (Harris et al., 2013). A centralized diet preparation operation is one in which diets are prepared in a single location. In addition, this location also tends to be the receiving and storage location for many of those feed ingredients. A decentralized operation can include a variety of approaches, but commonly includes diet preparation at a variety of locations throughout the zoo. There are some efficiencies to be gained by centralized diet preparation, especially if diet ingredients are reasonably consistent throughout the animal collection. This could include formulation of common mixes (nutritionally complete feeds, produce, etc), but also could simply include using the same individual item within multiple diets. Because the diets are prepared in a single location in close proximity to the inventory, the labor, equipment, and infrastructure efficiency can be maximized (minimizing cost associated with each aspect). In addition, if the inventory is maintained in a single location, it is much easier to manage and maintain than multiple storage sites throughout the zoo.

Employing a Professional Zoo Nutritionist

Perhaps the single most important thing in managing costs associated with feeding the zoo collection is to have a qualified individual focused solely on providing appropriate zoo animal diets in a cost-effective manner. This goes beyond simply a staff person purchasing the needed items and monitoring inventory. It goes beyond someone selecting the lowest-priced commercial carnivore diet they can find (or the most expensive), without an assessment of quality. It also goes beyond someone entering prices and nutrient contents into a computer program and following the output to offer a "balanced" diet to the collection. It goes beyond a visiting professional making inventory and diet recommendations once per year. It is someone to pay attention to matters at hand on a daily basis to ensure the nutrition needs of the collection are being met in a fiscally responsible way. It is someone trained to understand clinical diet management within the context of the bigger picture. It is someone who can make rational substitutions when certain ingredients aren't available, both for the good of the animal collection and the financial bottom line. It is someone to assess that an animal is being overfed, based on its condition, behavior, and consumption, and make the educated diet adjustment to not only save the waste, but also improve the health and welfare of the animal in question (also saving the "waist;" Rodriguez and Marques, 2010). Such value, although sometimes very concrete (in terms of cost savings), is also very abstract. Although not simply quantified, the value of a trained, qualified nutritionist on staff cannot be over-stated for all of these reasons and those detailed throughout.

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FOOD SAFETY TRAINING FOR KEEPERS IN DECENTRALIZED ZOO KITCHENS.

Devan Paulus Compart*

Lincoln Park Zoo, 2001 N Clark St Chicago, Illinois 60614, USA.

Abstract
A food safety program was developed for training keepers in a decentralized zoo kitchen system. Instruction covered food safety standards of the state of Illinois; reviewed information regarding USDA and AZA food safety standards; and provided keepers with action-based steps they could take to prevent food safety concerns. In addition, lead keepers had separate sessions to ensure food safety standards have been met by their direct reports. Keepers provided positive feedback regarding the sessions. Nutrition Center management conducting the training gained valuable insight regarding issues that interfere with proper food safety procedures and protocols within decentralized animal food kitchens.

Introduction
Zoos with decentralized animal food kitchens face challenges regarding food safety not generally observed in centralized kitchens. A number of keepers are handling animal food thus making them partially responsible for maintaining food safety at the zoo. However, most of these staff will not have undergone any food safety training. Having uncertified food handlers puts zoos at risk, as USDA and AZA standards for food safety may not be met.

In an effort to improve food safety throughout a zoo's decentralized animal food preparation kitchen system, a program was instituted to train all keepers in proper food safety methods. Further training was provided to lead keepers to overcome management issues associated with food safety.

Food Safety Training Goals
Three main goals were in place for the food safety training program. First, it was made clear that keepers handling animal food are partially responsible for maintaining food safety in a zoo. Secondly, education of keepers on action-based steps was provided so they could take steps to minimize food safety risks in their routines. Finally, keepers were provided critical food safety concerns that would be under scrutiny during inspections conducted by USDA or AZA.

Training Sessions
Training was conducted in eight identical sessions with small (4-6) and large (7-15) sized groups of keepers. The initial three sessions were provided to keepers located in a single animal building, while the remaining sessions were made up of keepers from various animal buildings throughout the zoo. This was done to ensure all keepers were able to attend training sessions regardless of their schedule.

Training included a 35 minute PowerPoint presentation conducted by the Nutrition Center Manager of Lincoln Park Zoo. Keepers were encouraged to actively participate in the presentation by asking the instructor questions.

Training Subject Matter

The first portion of the training session covered Nutrition Center operations. This topic included the inventory ordering process, as well as general tasks completed by Nutrition Center staff. The goal of this component was to familiarize keepers with the food safety steps taken within the centralized kitchen before it was forwarded to them. Information was included about ordering from reputable vendors, inspecting orders upon arrival, and inspecting food products before sending them to decentralized kitchens.

The second portion of the training covered basic food safety information using restaurant standards of the state of Illinois. Topics included preventing bacterial, chemical, and physical contamination; safe facilities; proper food storage and handling; and proper thawing and cooking methods. This portion of the training was designed as action-oriented, providing keepers with specific steps that they could take to reduce food safety risks. Examples of action-oriented steps were provided to the keepers, including wearing latex gloves over Kevlar gloves; working with small portions of raw meat at a time to minimize exposure to non-refrigerator temperatures; and sanitizing counters between working with produce and meat.

The third portion of the training covered the identification of unsafe food products including categories of produce, meat, fish, forages, complete feeds, and ready-to-eat products. Keepers were provided with action-based steps that they could take when food safety concerns were identified, such as taking photos of the unsafe products and contacting the Nutrition Center manager.

The final portion of the training covered kitchen inspections using information from the kitchen inspection checklist utilized at Lincoln Park Zoo. Prior to this training, keepers had little understanding regarding what critical steps needed to be taken in order to have kitchens compliant with USDA and AZA standards; thus, decentralized kitchens frequently failed to be compliant during routine inspections conducted by the Nutrition Center manager at Lincoln Park Zoo.

Observations

Positive feedback regarding the training sessions was obtained from both large and small groups of keepers, as well as sessions conducted with one house of keepers versus multiple houses of keepers. However, it was observed that small groups tended to interact with the Nutrition Center manager more frequently and ask more direct questions. Small groups also posed a wider range of questions that covered not only zoological food safety, but also human kitchen food safety. Training offered to keepers working in the same decentralized kitchen resulted in keepers asking questions more directly related to specific food safety issues they face thereby allowing the Nutrition Center manager to provide directly applicable help and resources.

Lead Keeper Training

In addition to general food safety training, supplementary training sessions were completed with lead keepers with the goal of helping them identify strategies to ensure direct reports were compliant with food safety protocols and procedures. This training was conducted in two sessions with 3-5 lead keepers in each session. Training took place in various decentralized kitchens throughout Lincoln Park Zoo. In each kitchen, the lead keeper managing the kitchen described methods they utilized to ensure food safety protocols and procedures were met. This demonstration was followed by a discussion among training participants regarding the pros and cons of various management methods, as well as discussion regarding the feasibility of implementing the various methods in their own kitchens. Overall, these training sessions were primarily lead keeper led with minimal input from the Nutrition Center manager other than session coordination.

Lead keepers expressed positive feedback regarding their personalized training session. Many found that they were able to gather new ideas that could be implemented in their own kitchens. In addition, it was an opportunity for lead keepers to discuss how various management techniques could be applied in the unique kitchens they manage. It was noted that most lead keepers felt that lack of support from upper management was a major hindrance in their ability to properly manage food safety in their kitchens. Further, several lead keepers stated that lack of keeper interest in food safety resulted in failure to follow-through on food safety protocols and procedures.

Conclusions

Overall, food safety training provided to keepers in a decentralized kitchen system resulted in positive feedback from training participants and increased interest in food safety. In addition, providing lead keepers with additional training allowed for critical feedback regarding food safety policy and procedure implementation issues in decentralized animal food kitchens. It is recommended that zoological institutions with decentralized kitchens provide training to all zoo keepers handling animal food as a means of reducing food safety risks and ensuring USDA and AZA food safety standards are met.

THE TOOLS WE USE: OUTFITTING YOUR OPERATION FOR EFFICACY AND EFFICIENCY

Matthew A. Brooks, Ph.D.[1]* and Michael T. Maslanka, M.S.[2]

[1]Oregon Zoo, 4001 SW Canyon Rd., Portland, OR 97221, USA
[2]Smithsonian Conservation Biology Institute, National Zoological Park, Center for Animal Care Sciences, Department of Nutrition Science, 3001 Connecticut Ave., Washington, DC 20008, USA

Introduction

Outfitting your nutrition operation can feel a bit like building an arsenal with which you can draw from to fight the good fight that is feeding your animals. However, what you put in your arsenal will depend entirely on your goals as a department and the space and capacity you have available. Wherever your operation lands on the centralized/decentralized commissary continuum, it will be essential for your operation to have good quality equipment to help you provide the best diets for your collection in the most effective way possible. The intent of this paper is to help you and your institution make the right choices about what equipment will best suit your operation and your animals.

Assessing Your Needs

The following items need to be considered when purchasing equipment:

1) Institutional needs
    - Animal/Species Needs
    - Safety
    - Sanitation
    - Collection Size
    - Centralized/Decentralized Operation
2) Space/Resources Available
    - Do you have room in your kitchen or to store it?
    - Can it be easily installed?
3) Staffing Requirements
    - Will it take more time?
    - Will it make work more efficient?
4) Funding Available
    - Will it reduce waste/staff time, thus pay for itself?
    - What is the initial investment?

Outfitting Your Operation

The following is a list of equipment that institutions currently use and/or have found to be useful and, in many cases, necessary to their operation. For most products, there are several companies which you can choose from, and this paper is not meant as an endorsement of any one particular brand. We will, however, give some specific examples to help illustrate certain items on the list. Above all, when you choose a product, make sure that the product you choose is a good quality product that will withstand what you are about to put it through.

Kitchen - Food Prep

- *French fry cutter (manual)* - Simple use of a lever arm and a blade grid allows for the quick processing of long cut sweet potatoes, apples, and other produce. Made from metal, it is easily dissembled and hand-washed or sent through a dishwasher (watch your material, as not all types are dishwasher safe). The cutter can be wall mounted above a small table (allow for reach-across access, 18-24" wide) for placing the container/bin underneath the cutter itself. Because this is completely human-powered, ergonomics (height and reach) are crucial. Moving parts can be lubricated with a food grade oil. This is a relatively inexpensive item to purchase and install.
- *Vertical cutter/mixer* - A vertical cutter mixer (VCM) allows for a wide range of tasks to be performed with the same piece of equipment. In addition, attachments can be purchased to take greater advantage of the platform. The main bowl of the cutter mixer can be used for a variety of kitchen needs from processing biscuits and pellets into a powder to making meat mixes, formulas, and gruel-type diets. A side attached food processor performs all of the same functions as a stand-alone processor, but is powered by the core of the VCM. These can be expensive pieces of equipment, but used models are often available affordably. The VCM requires a dedicated floor footprint, and is quite heavy (most have wheels on one side for a tilt/move option).
- *Blender* - Blenders in the kitchen are used for a variety of tasks from powdering small amounts of biscuit or pellet to making gruel-type or gel-type diets. For zoos that tube feed often, it allows for mixing multiple ingredients such that they will readily flow through various gauge tubes. There are numerous models and designs available from industrial stainless steel (tend to be more expensive) to nearly disposable, small motor, and/or coffee grinder types. This is one of the most common equipment types found in many zoo commissaries.
- *Microwave* - Microwaves can allow for quick heating of water when a stove or other method is not available. A microwave also can allow for quickly steaming vegetables as needed. It should be noted that a microwave in the commissary is usually animal food only, and if this is the case, it needs to be labeled accordingly.
- *Stove* - A stove can be a useful addition in the commissary. It allows for all of the same tasks as the microwave, but can also provide for other non-microwavable tasks (i.e. hard boiling eggs, etc). In addition, if meat needs to be cooked (i.e. chicken), often the stove is a more thorough and efficient method.
- *Band saw* - A band saw designed for cutting meat, bone, and other food items can be an invaluable tool for reducing waste if you have the facilities to accommodate it. It can be used to cut frozen meat and fish to sizes that allows for more rapid, even thawing, and it enables the thawing of partial packages to prevent thawing of excessive product due to packaging size. This item can also be the most dangerous item in your facility. Commercial kitchen saws require not only floor space for the machine, but generally they are hardwired to an electrical box which requires a specific zone of safety clearance. The machines can be custom fitted with electrical plugs, but they require higher voltage than a normal outlet and the plug will need to be waterproof for cleaning. Further safety requirements include a secondary shut off on the electrical box that can be "locked-out" during cleaning and maintenance. The saw does not necessarily need to be located in the kitchen if size or electrical requirements are a hindering factor. However, cleaning the saw requires a lot of water, so access to a hose and proper floor drains are a must. When

working with this item, proper personal protective equipment (PPE) such as a face shield or other eye protection and gloves need to be worn.

- *Knives, Sharpeners, Gloves, and Accessories* – These are likely the most used tools in the kitchen. It is very important to make sure the right knife is being used for the right job. Even more important is that these knives need to be kept sharp at all times for safety. A good combination of knives to keep in the commissary include, but are not limited to, 8-10” Chef’s Knife, 4-6” Utility Blade, Boning Knife, Kitchen Shears, Paring Knife, etc. Select knives that are NOT serrated as they are harder to sharpen and serrations are used for sawing, not cutting. Other items to include in the kitchen are: a steel, which does not sharpen the blade only straighten or hone the edge, and a sharpening stone. The option is also available to employ and outside company which will sharpen knives on a regular basis. Storing knives is best done in a working kitchen with a magnetic knife strip attached to a wall. This prevents the blades from getting dulled in drawers or counters, and it keeps anyone from accidently getting injured by misplaced knives. Cut resistant gloves can help reduce cuts in the kitchen. For working in the kitchen, lighter weight gloves composed of materials such as Spectra®, Kevlar®, etc. allow for greater dexterity and flexibility with decreased hand fatigue. Some gloves come color-coded by size, and are made with microbial resistant material. All gloves need to be washable to prevent cross-contamination and bacterial growth. All cut-resistant gloves are rated by the amount of weight required to cut through the material, so make sure that the glove rating is sufficient for the level of protection you may need.
- *Anti-fatigue mats* - Mats are designed to relieve foot pressure and reduce the strain on the lower back and legs of those who have to stand and work in one place for extended periods of times. For the kitchen environment, mats made with anti-microbial rubber prevent slippage on wet floors and can be cleaned and sanitized on a daily basis. Perforated mats provide drainage of liquid and debris. Solid mats have the disadvantage of holding in moisture if the mat is placed on a wet floor; therefore perforated mats are preferred for the wet environment of the kitchen.
- *Scales* - The needs of an institution will vary when it comes to the size and number of scales an operation requires. Although the initial cost of a quality scale is higher than food scales found at the grocery store, the capabilities and life span of these products are well worth the price. The important things to look for in a scale are sensitivity (how precise is the scale), durability, and size. Scales can measure within a range from less than a mg up to many kg. The main workhorse of many kitchens is a gram scale with a range from 0.1 g to 2 kg. Generally, as scale weight capacity increases, sensitivity decreases. Having different sized scales for different tasks may be necessary. “Wash-down” scales are made for wet environments and can handle a wide array of temperatures if they need to be used in the cooler or freezer. Smaller scales may require a separate cover to help keep them dry, clean, and functional (based on sensitivity). A useful addition to scales used in the kitchen is an Infrared Tare Function that will zero the scale by just holding a finger over the sensor. This helps prevent cross-contamination by keeping dirty hands from touching the device. The size of the platform is also important. If a container is too large for the platform, it can cause the scale to be unbalanced, leading to inaccurate weights. When purchasing scales, also find an external company that is able to provide annual calibration and regular maintenance. These companies can also recommend scales that are better quality and more cost effective to repair when broken.

- *Feed Mill Grinder* - Also known as size reduction equipment. These range from large hammer mills all the way down to coffee grinders. They can be useful for both making diets and preparing samples to send for analysis. The ability to grind a dried feed product expands the feeds available to specialized feeders that do not have the ability, mouth size, or dentition to eat larger pellets. Powdered commercial diets can be easily mixed into chopped fruit and vegetable diets or gelatins to allow for uniform distribution in the diet. Things that need to be considered when looking for a grinder include 1) Material to be processed (size, coarseness, moisture content) 2) Desired particle size 3) Throughput. These will determine the size and style of grinder needed along with the screen size, RPMs, and power requirements.

Kitchen - Organization

- *Flip books* - There are a variety of ways to organize "recipes" within the kitchen from the modern tablet/touch screen options (discussed below) to the more basic folders and flip books. Flip books are set up much like three-ring binders on a stand, but are specific in design and can hold many pages (some come in expandable "sets" of 20, and can be combined up to 100 or more pages). Flip books allow for easy access to diet or recipe cards, can be easily edits and cleaned, and are a simple approach to organizing diet preparation activities in a variety of conditions.
- *Color coded cutting boards* - Cleaning and sanitizing cutting boards and utensils between jobs is imperative. Another precaution that can be taken is the use of color coded cutting boards (and other utensils), based on the job. Meat and fish handling utensils can be colored coded differently than those used for produce or other purposes. If this additional failsafe is used, it is important that the color code is communicated and understood across the staff using the items.
- *Labels* - Keeping items properly labeled is an essential part of any food service operation. Labels in the kitchen need to be able to handle both wet and cold environments, but also be able to be removed easily for cleaning. Although there are food service labels available will dissolve in 30 seconds of running water and are environmentally friendly, if they are used for delivering diets in a rainy environment (say for example Portland, OR), the difficulty becomes that they will dissolve before they ever reach the delivery area. Thermal printed labels will not dissolve and the lack of ink means no smearing and no purchasing ink cartridges. However, these labels require a special printer and the labels themselves can be more expensive than sheet labels.
- *Tablets* - Tablets are becoming more cost effective by the day. Having a tablet in the kitchen can replace paper diet books. A tablet connected to a wireless network will allow diet changes and ordering to be more automated, thus reducing the amount of time needed to implement changes and reducing the likelihood of mistakes occurring, such as labels not getting corrected or diet cards not getting changed. It would allow a nutritionist across the zoo to update diet cards in real time. Further, most tablets have cases to protect them in wet and rugged environments while also providing a built in stand. Ordering can be done on a tablet while doing inventory, and if needed, they can be attached to a keyboard or docking station to provide an extra computer for interns or staff.

Food Service Sanitation

- *Dishwashers* - There are multiple types of dishwashers available, based on the size of the operation and the numbers of dishes to be handled daily. For most operations, due to daily use, an industrial model of some type is most prudent. For smaller operations, a single compartment, short-cycle, one-rank-at-a-time could work just fine. For zoos with centralized operations where many dishes are returned to the commissary at once, a conveyor type dishwasher may be more useful. This allows for dishes to be loaded into racks and fed into the machine at one end, as clean/sanitized dishes are pushed out the other end. More space is needed for these conveyor type dishwashers, but they allow greater throughput for dish-intensive systems. Regardless of dishwasher set up, it is important to consider not only space (places for dish racks to run out of a conveyor, or places to store clean dishes, etc), but also humidity control. Dishwashers create a lot of humidity (steam, moisture), and appropriate ventilation is required for employee safety and sanitation. In cases where heat sanitizing is preferred over chemical sanitizing, the dishwasher may need to be affixed with a booster heater to increase the water temperature from the standard hot water heater in the operation to sanitizing temperature.
- *Sinks* - When a dishwasher isn’t an option, it is best to invest in a three or four bottom system. This system works by having a three compartment sink plumbed together in series with the first compartment closest to the drain being the wash sink, the second sink being a rinse, and the third sink being a sanitizer. Each sink is filled at the start of each day and used throughout the day. Water is changed out in each compartment as needed throughout the day. The design allows for each sink to be emptied in succession starting with the wash sink, this will then wash, rinse, and sanitize the plumbing daily. Another consideration is whether to add a fourth compartment as a pre-wash sink. If possible, having a sprayer wand along with a garbage disposal on a sink prior to the wash prevents the need to continuously change out the wash water due to debris build-up.
- *Hoses* - A water wand sprayer attachment on a sink can do a great deal to help pre-wash any dishes. Most industrial sinks can be fitted to accommodate a sprayer. A reel hose installed in the kitchen can help with cleaning on a daily basis. This requires proper drainage in the kitchen to allow for large volumes of water, and the kitchen and any equipment must be able to handle being hosed down. This prevents the use of any wood shelves. Generally walls need to be concrete or covered in fiberglass reinforced plastic (FRP) panels. For this to be effective, the hoses need to have hot water lines run to them, and the facility must have a proper air handling system to remove any excess humidity.

Materials Handling

- *Hand trucks* - Often, when dealing with individual bags of feed or larger bales of hay (or multiple types of one-offs), it is easier to move product with a hand truck (or dolly). These can have a straight platform, or can have forks held on a spring release (to move into small plastic pallets). The small plastic pallets are made to hold 10 bags of feed, stacked vertically, and be used to maximize space, if a standard 4x4 wooden pallet is not practical for the space or operation.
- *Pallet jacks* - A manual (non-motorized) pallet jack is worth its weight in gold. It is inexpensive and can be easily moved with a forklift onto delivery trucks to move items loaded near the cab to the back for easier offloading. When selecting a motorized pallet jack, the load weight that needs to be moved and the grade of the building both need to be

considered. If a pallet jack is underpowered to go up a grade with a full load, it is a waste of money and a hazard in the operation.

- *Reach trucks* - Reach trucks (stand up pallet stackers or walkie stackers) are extremely useful when you have stackable racks that hold 4x4 pallets (wooden or plastic). These machines have an extremely tight turning radius and can fit down narrow aisles and alleys. Because the operator is standing up inside the machine, it also allows for better clearance and safety. Attention should be paid to the lift capacity of the truck itself, the forks down clearance (ensure access through your doorways), and ease of operation. If a building has the capacity for shelf storage of pallets, a walkie stacker can lift up to three levels and will be a space and back saving tool. If possible, invest the additional funds to opt for a side-shift model. This makes moving pallets into tight spaces much easier.
- *Tractors* - Based on the operation, tractors can be very useful pieces of equipment. If fitted with hydraulic arms, a set of forks can be used, similar to a standard forklift. Rear attachments allow for various mowers to be attached, for use in trimming or maintaining browse stands, harvesting fresh cut hay, moving hay (described below), etc.
- *Forklifts* - These vehicles can be essential to an operation if dock access is unavailable. If horizontal space is limited, vertical space may be a better option. When selecting a forklift, ensure that the model chosen is counterweighted properly to maintain stability when lifting heavy materials, especially when extending the full height of the mast. Forklift operation also requires proper operation and safety training, and many facilities require licensing or certification.
- *Forklift/Tractor Hay Handling Attachments* - If an institution houses elephants or a lot of hoofstock, handling hay can be a large part of the job requiring several people to check it, stack it, and deliver it. The amount of people required for such a task can be decreased to one if the proper equipment is used. If your institution uses round bales, a hay spear attachment is a necessity. Although these are available for forklifts, this attachment on a tractor allows you to move hay to different areas of a pasture or paddock with relative ease. When dealing with square bales, a hay grapple attachment on a forklift not only allows one well trained operator to unload and stack multiple tons of hay quickly. If floor space is at a premium and head-space is not, then this is an option. The drawback to this system is that this cannot be done with a light-duty forklift, and the counterweight on the forklift must be enough to handle the weight of the hay at the intended height of the stack.
- *Chain saws, loppers, and hand saws* - There are a variety of methods used for harvesting browse and bamboo. Hand saws and loppers are common manual-type harvest tools. Gear-driven loppers reduce the felt force needed to cut larger diameter branches and bamboo. Hand saws that can also be attached at the end of pole pruners allow for the dual use of the saw itself. For larger woody browse and thicker bamboo, there are automated options that increase the efficiency of the process, but also increase the risk. Electric (battery powered) pruning saws (saws-all type) are very useful for these operations. Risk is somewhat minimized with an electric, fixed blade saw, but appropriate PPE is still necessary. Chain saws (whether gas or electric) are potentially the most dangerous tools we can use (in addition to a band saw). Appropriate training for operation and safety is required. That said, when in the hands of a competent operator, a chain saw can quickly and efficiently handle bamboo and browse of all sizes.

- *Dock leveler/Above grade dock access* - Many of the items we use are shipped in bulk and arrive in the back of a trailer. Ensuring there is adequate space for the trailer to access the receiving location in imperative, but once in place, it is important to have a way to get the items safely and efficiently from the trailer to your commissary. Having above grade (trailer height) receiving space benefits not only the operation, but also the delivery drivers. Associated with that dock access is having a dock leveler to ensure ease of unloading once the trailer is in place. This links the dock to the trailer in a safe way to allow on- and off-loading. If a dock leveler is not available, a specifically-designed steel plate system can allow access between the dock and trailer (although is not ideal).

A Final Word

"An elephant for a quarter is only a deal if you need the elephant and have the quarter." There are many options available to make commissary operations efficient. Selecting the best options for each operation is based on needs (current and future), existing infrastructure, staffing, and resource availability. Ensuring a good fit between the tools or equipment and the nuances of the existing operation is imperative so that resources (especially financial) are utilized to their greatest benefit. Perhaps more useful than the tools and equipment described herein, is the application of a creative approach to their efficient and effective use within each individual and unique operation.

Examples of different operation set-ups

Oregon Zoo (Centralized) - 1.5 FTE Kitchen Staff & 2,000 Collection Animals

- Bale Grab attachment for forklift - With our limited staffing we could not handle hay unloads without it.
- Hobart Meat Band-Saw - Our model has been running for decades. Used for sawing meat chunks, fish, calf carcasses, etc.
- Hammer Mill - Our model is donated from an old feed mill. Used to grind up whole bags of feed for use in diet mixes (bat chop diet).
- Hyster Forklift - Our model has extra counter-weighting to lift bales 3 stories in our hay barn.
- Pallet Jack - Manual and Motorized. We use the manual one to unload truckfulls of baled bedding and motorized for everything else.
- Hobart Chopper - We only have a smaller version that we use for chop.
- Ohaus Scales - Our diet scales have the infrared zeroing capability so you don't have to touch the scale to zero it, just hold your finger over it. Keeps the scale cleaner. We have other scales of different capacities 2.1 kg - 50 kg. Sensitivity is not as great as the scale capacity increases. We have a local scale company calibrate all the scales yearly
- Flip Books/Tablets - We currently use flip books, but we just purchased Microsoft Surface 3 Tablets for diet books. Got the Urban Armor covers for heavy duty use.

Busch Gardens - Tampa (Centralized) - 5 FTE Kitchen Staff & 3,000+ Collection Animals

- Robot-Coupe Blixer 60 – grinds feed (insectivore powder, primate biscuits, etc.). Probably could replace the Hobart if we didn't object to mixing meat and dry goods in the same equipment.
- Robotcoupe CL60: This is a food processor that slices and chops produce into different size squares or slices (disc slices or coleslaw type stuff). We use it every day to make the

different salads, fruit mixes, and fruit bat mixes. We spend about 2hrs/day with this machine. Could not function without this (or, would need another 2 employees).

- Hobart floor model mixer: This we use every day to make our meat mix (meat, fish, and flamingo powder). We also use this machine to mix our gels. It works great and we have been using it close to 40yrs.
- Label maker - Brother P Touch: Use this all the time. They stick well to items in the cooler, fairly well to ones in the freezer.
- Sartorius Scales: use these every day, all day. Couldn't live without these. Seems like a really good name brand that we have had very few problems with.
- CAT Pallet jack: This is used several times a week and has been old reliable for the last 30 years
- Chef's choice knife sharpener: works great
- The most used machine in the kitchen is the BOSE Radio: Used every day, all day. Can't stress the importance of having a good sound system. Just do it!

National Zoo & SCBI (Centralized) - 9 FTE Kitchen Staff & 2,000 Collection Animals

- Meat saw
- Microwave
- Multiple blenders
- Multiple scales
- Knives
- Food processors of multiple types (NOT a VCM, though)
- Stand up reach truck, Stand-on, Walk behind electric, & manual pallet jacks
- Chain saws, electric fixed blade saws, hand saws, pole pruners, weed eaters with brush blades
- Flip books

Cincinnati Zoo & Botanical Gardens - 4 FTE & 2500 Collection Animals

- Fork lift, Electric, & Manual pallet jack
- Scales
- Steamer
- Meat Refrigerator that we term "The Thawer" – I have not seen this piece of equipment anywhere else

Taronga Zoo

- We're really fond of the Robot Coupe products, particularly the attached Workstation. We have a smaller model but this workstation is ideal. The only thing I would suggest is rather than getting all the different discs, get multiples of the ones you would use most. The company is really good at mixing and matching to meet your needs.
- The bale grab has been very useful at our safari zoo, too.

TOOLS AND TECHNIQUES FOR SUCCESSFULLY GRINDING FISH FOR TUBE FEED FORMULAS

Juliet Eckert

Georgia Aquarium, 225 Baker Street, Atlanta, Georgia 30313 USA.

Abstract
Grinding fish in a blender is very complex because the fish often need to be processed to some degree and there are many choices of blenders. Suggested blenders to use include Waring blenders of various sizes, homogenizers, or smaller, but more affordable, Magic Bullet® or NutriBullet®. Using a meat grinder, chopping, filleting, skinning, and successive blending are all methods of processing the fish or other seafood to break it down for optimal blending. There are advantages and disadvantages for every blender and technique. The ratio of fish to water is also important, as enough water is needed to homogenize the formula and to make the formula fluid enough to easily glide through the various sized feeding tubes. Considerations need to be made for the size of the feeding tube, the amount of formula needed, and how much water needs to be added (generally, 70% fish to 30% water) to achieve the desired consistency and/or caloric value. With practice and properly maintained blenders, creating formulas using seafood products can become easier. Knowing which technique and tool to use is just the first step, but practicing helps to refine the skills needed to successfully grind fish for tube feed formulas.

Introduction

At the Georgia Aquarium, we have a variety of blenders and other tools that we use to process fish or seafood in the event that we need to tube feed an animal. However, larger fish, such as herring, cannot simply be added to a blender with water and come out homogeneous enough to easily pass through feeding tubes. The fish need to be cut, likely skinned, and filleted. Some fish and other seafood need more water than others to reach a proper consistency (e.g. shrimp). There are other considerations when one is preparing a tube formula for smaller fish, such as seahorses or discus. The tubes used are very small (e.g., 3.5 and 5 fr. red rubber catheter tubes) and the formulas need to be fluid enough to easily pass through these tubes. Smaller blenders, such as a homogenizer (VWR model VDI 25 S41, see Figure 1) can be helpful with this, but there are problems associated with them as well. There are a couple of commercial home products the Aquarium uses to successfully create a variety of tube formulas, the Magic Bullet® and the NutriBullet®. We also have a manual meat grinder for grinding whole fish and two large blenders, a Waring commercial blender (2 L capacity, model HGB160, which is similar to the currently available model HGB150) and a Waring Heavy Duty blender (4 L capacity, model CB15) for handling larger batches of gruels (see Figure 2). Over the years we have developed several techniques to deal with the challenges of grinding fish or seafood.

Discussion

The type of fish to be used in the gruel, the ultimate consistency required (e.g. what animal is the formula for/what size tube is being used), and the caloric content are going to be the top considerations when deciding which technique to use in processing the fish. Other factors of importance are amount of gruel, time the gruel will be used, and other ingredients to be added.

We never use less than a total amount of 100 g unless we are making a gruel that will be mixed with the homogenizer. Anything less than 100 g does not typically grind well in the Bullets because the blades cannot grab enough material to mix and chop sufficiently. Our nutritionist sets the ratio of fish to water; this is generally 70% fish to 30% water based on consistency and caloric content. However, there are occasions where more water needs to be added in order to obtain a fluid consistency (but always remember to keep track of how much water is added in order to recalculate the actual ratio). Shrimp tends to require more water than other foods in order to become more fluid.

Most tube feed formulas are made within an hour or two of feeding. Some do require more advanced preparation due to personnel or other time restraints. All tube feed formulas are used within 24 hours. However, there are certain fish and seafood products that tend to solidify faster and would require them to be made as close to feeding time as possible. These include shrimp, and some blister pack fish formulas, such as discus formulas (various brands). Also, if any gel powder is added (such as Mazuri® Aquatic Gel Diet), that will also cause the gruel to thicken, so feeding out as soon as possible is preferred.

Some formulas may need to be heated up in a water bath before being fed out. An example of this is milk replacement formula. Sometimes a fish formula, after sitting in a cooler for an extended time, will invariably thicken and will need to be thinned with water.

The following are four techniques for processing fish that will work in different situations, followed by the disadvantages of the Magic and NutriBullets® and the Waring blenders.

Chopping into smaller pieces

Some fish can be ground whole (e.g. silversides, *Menidia menidia*). However, for more effective blending, it is best to chop the fish up before attempting to grind. This is best done with fish that are not completely thawed. The NutriBullet® can handle this in small batches (100 g to 300 g total gruel). Tilting the NutriBullet® or Magic Bullet® at a 45° angle can improve the mixing capability.

Skin and fillet

Larger fish will often need to be skinned and filleted before being ground in a blender. Head, tails, and fins also do not grind well and should be removed. The skin and spine will not mix and therefore block up the tube when feeding (see Figures 3-5). Using a strainer to remove these after blending was an ineffective use of time and only lessened the amount of total useable gruel. Using fish that are only slightly thawed makes it easier to simply pull the skin off.

Successive blending

When using the larger Waring blenders, sometimes successive blending is useful to achieve a finely ground gruel. The larger blenders may not be able to homogenize the fish finely enough for some purposes. Therefore, additional time in a smaller blender, such as the Magic Bullet®, is necessary. The disadvantages to this method are that you lose volume and nutrients when transferring from one blender to the other.

Meat grinder

Another way to break the fish down before grinding is to use a meat grinder. The meat grinder takes the whole fish and breaks it down into a fish paste that a blender can easily process with minimal water. However, fins and parts of the head sometimes do not make it all the way through the grinder, not to mention the rest of the fish that is left in the grinder that cannot be cleaned out easily (without rinsing). This method will be able to use the most of the fish, which may be preferable if sample analyses are done using whole fish.

Homogenizing

A homogenizer can be used to finely mix small samples of seafood products. This piece of laboratory equipment is often used to homogenize samples of tissues, plants, soils, and others. Use the homogenizer when making gruel for small fish because making a 100 g formula for such a small animal is excessive. Use a 50 mL centrifuge tube to hold the seafood product (e.g. mysis) and water and then follow the manufacturer's instructions for using the homogenizer. The problem with homogenizers is heat generation. The seafood product thaws faster because the amount is so small; it does not stay cool as long as the larger fish formulas. The intense mixing of the homogenizer generates enough heat; it could be damaging vitamins and denaturing proteins. It is important to limit the mixing time to only as much as is needed (typically only 30 seconds or less) to prevent as much heat degradation as possible. Another disadvantage to the homogenizer is its cost, which can be over $1,000.

Disadvantages of the NutriBullet® and Magic Bullet®

There are some drawbacks to using the NutriBullet® and Magic Bullet®. One, they can be expensive (though not nearly as expensive as the Waring blenders). NutriBullets® cost $89.99 from the original manufacturer (www.nutriliving.com), and come with a few things that are probably not going to be used. Individual pieces are available (bases, mixing cups, and cross blades) from eBay and Amazon. Magic Bullets® are currently being offered for $99.99 for 2 sets from the manufacturer (www.buythebullet.com). However, the set comes with many accessories that are useless (party cups and shaker tops) for zoological operations. The milling blades that come with both the NutriBullet® and Magic Bullet® sets can be used for some seafood items (such as pacifica krill), but in general they will not be able to grind up fish to the same degree as the cross blades. There is an ice chipper blade available for the Magic Bullet® that can be purchased separately.

Another disadvantage is that they tend to frequently break with high volume commissary use. Many of the problems associated with the Magic and NutriBullets® stem from the fact that the conditions of an aquarium's commissary are generally harsher than what they are designed for. The water environment is harsh on the Magic and NutriBullets®. If the blades are not fully tightened around the cup, when they blend, they leak, which eventually causes damage to the motor. The way the Magic and NutriBullets® are constructed makes them more difficult to clean than the Waring blenders. The gaskets in the blades are difficult to take out of the Magic Bullet®, but easier in the NutriBullet. The bases are more prone to water damage than the Waring blender bases, so more care needs to be taken when cleaning them. The constant washing and sanitizing takes a toll on the bushings of the blade assembly; they may seize up and a new blade will be needed (however, there have been improvements with the NutriBullet®). As previously stated, there are individual pieces, as well as spare parts for the blades, available on eBay and Amazon.

Disadvantages of the Waring blenders

The models previously stated can only process larger quantities of fish; however, Waring makes a smaller 1 L capacity blender,and their laboratory blenders have attachments for the 1 L blender that can grind even smaller quantities (12 - 250 mL). However, Waring blenders are very expensive. The models are between $250 and $1,000. The attachments for the the 1 L blender are around $300. These blenders are more durable than the Magic and NutriBullets®; however, when they do need replacement parts, they can be costly. As an example, a replacement blender container for the 2 L blender was nearly as much as the actual blender cost. The manual states that the blade assembly has a life expectancy of about 500 hours of running time depending on operating conditions (and grinding fish can be tough on grinders). Replacement blender parts can be purchased but a skilled technician is required; otherwise the blender must be sent to a Waring service center, or a new blender container must be bought.

Waring blenders will also generate heat when running for extended periods (although they should not be running continuously for more than three minutes). Even after repeated bouts of blending, fish formulas may warm up, which may cause heat degradation of proteins and vitamins. If a formula does not have any cold fish in it (such as milk replacement formulas), the use of cold water will only minimally offset the heat generation caused by the friction of the blades.

Conclusion

Fish can be successfully ground into a tube formula for the treatment of animals. Extra steps need to be taken to ensure the homogenization of the fish. Properly maintained blenders or other tools, such as meat grinders, are essential for this as well. There are a few disadvantages to each blender and each technique is only useful for certain applications, however, practice and experience will eventually optimize the process.

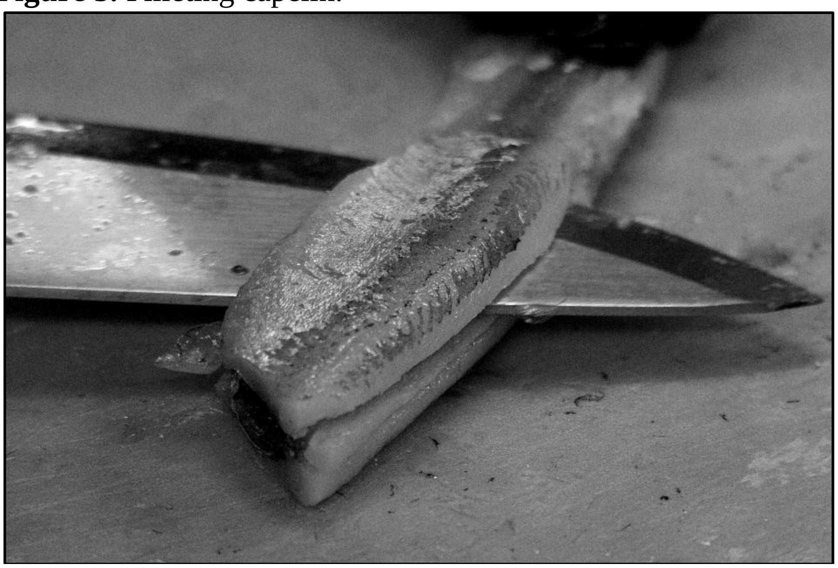
Figure 1. An example of a homogenizer, VWR model VDI 25 S41

Figure 2. From left to right: Waring Heavy Duty CB15 4 L capacity, Waring HGB160 2 L capacity, NutriBullet® with a tall cup next to it, Magic Bullet® with a tall cup next to it, 10 lb manual meat grinder.

Figure 3. Skinning a pacific herring fillet.

Figure 4. Skinning a capelin.

Figure 5. Filleting capelin.



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EVALUATION OF VITAMIN A STATUS AND DIAGNOSIS OF HYPOVITAMINOSIS A IN AMPHIBIANS

Allan P. Pessier, DVM, Dipl ACVP[1]* and Carlos E. Rodriguez, DVM, Dipl ACVP[2]

[1]Wildlife Disease Laboratories, Institute for Conservation Research, San Diego Zoo Global, San Diego, CA 92112-0551 USA;
[2]Department of Animal Health, Disney's Animal Kingdom, Orlando, FL 32830 USA

Abstract

Without supplementation, insect-based diets for amphibians and reptiles are known to be deficient in nutrients such as calcium and vitamin A (Livingston et al., 2014). In the last decade, hypovitaminosis A has been recognized as a limiting factor for amphibian conservation programs that must successfully maintain and breed endangered species for ex situ rescue, survival assurance, and reintroduction programs (Pessier, 2014; Rodriguez and Pessier, 2014). Empirical treatments, new approaches to dietary supplementation of insects (especially carotenoids and techniques for more effective gut-loading), and experimental studies have been useful for improving our understanding of this condition.(Brenes-Solo and Dierenfeld, 2014; Dugas et al.,2013; Livingston et al., 2014; Pessier, 2014). However, research and clinical management have been hampered by practical considerations (e.g., it is difficult to get sufficient samples from very small frogs), limited basic information on vitamin A metabolism in amphibians, and a lack of standardization in diagnostic methods (Glugston and Blaner, 2014; Rodriguez and Pessier, 2014). A presumptive diagnosis of hypovitaminosis A in amphibians will often be made by a pathologist observing squamous metaplasia (SM) in a normally mucus-producing or ciliated epithelium (Pessier, 2014; Rodriguez and Pessier, 2014). Although the tongue is the most common anatomic site for SM (i.e., short tongue syndrome), it is also recognized in the oropharynx, esophagus, ureter, reproductive tract, and cloaca. It is important to note that SM is not observed in every vitamin A-deficient animal, nor is it consistently observed in every anatomic site (Rodriguez and Pessier, 2014). Collection and histologic examination of a range of different tissues is suggested for every amphibian necropsy. Measurement of vitamin A levels in serum or liver is encouraged to confirm a diagnosis of hypovitaminosis A, but there are important pitfalls including awareness of what is measured and reported by different laboratories (retinol+retinyl esters or simply retinol) and the need for proper collection of samples (autolysis and light exposure influences results; Glugston and Blaner, 2014; Rodriguez and Pessier, 2014). Interpretation of vitamin A levels can be frustrating because of a lack of validated reference ranges in amphibians, wide variation in "normal" upper levels between individuals and species, and physiologic maintenance of serum retinol levels until deficiencies are advanced (serum retinol is not linear in relation to deficiency; Berkvens et al.,2014; Glugston and Blaner, 2014; Rodriguez and Pessier, 2014; Sullivan et al.,2014). However, low vitamin A levels (e.g., < 5-10 µg/g retinol in liver) should always raise suspicion of deficiency.

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UNDERSTANDING THE INTERACTIONS OF DIET AND LIGHTING ON FROGS AND THEIR SYMBIOTIC BACTERIA TO IMPROVE EX SITU HUSBANDRY OF AMPHIBIANS

Rachael E. Antwis, PhD*[1], *Christopher J. Michaels, PhD*[1,2], *Richard Preziosi, PhD*[1], *and Andrea L. Fidgett, PhD*[3]

[1]University of Manchester, Oxford Rd, Manchester M13 9PL, UK;
[2]Zoological Society of London, Regent's Park, London NW1 4RY, UK;
[3]Chester Zoo, Caughall Road, Upton by Chester, Chester CH2 1LH, UK

Abstract

Amphibians are undergoing massive population declines in the wild, in part due to infectious diseases including chytridiomycosis, caused by the fungal pathogen *Batrachochytrium dendrobatidis* (Bd). In response, amphibian populations are being maintained in ex situ breeding programs while viable treatments for chytridiomycosis are developed. One potential action involves the use of symbiotic bacteria from the skin of amphibians; however multiple factors likely affect the success of such probiotic applications. Disturbances in the natural microbiota of amphibians may alter individual susceptibility to pathogens and disrupt any probiotic treatments that may have previously been applied. Studies have found wild populations of amphibians show large variation in bacterial communities according to host species and location, thus conservation efforts may require baseline data for specific species and populations. The bacterial community of an individual is influenced by its biotic and abiotic environment, and amphibians in the wild receive relatively high exposure to bacteria through environmental and conspecific interactions. Conversely, the captive environment likely provides lower environmental heterogeneity and reduced conspecific interactions, potentially resulting in lower exposure to bacteria. Therefore there may be species- and institution-specific responses of the amphibian microbiota to the captive environment. Varying dietary and environmental conditions provided in captivity may also lead to differences in the microbial community, potentially leaving captive amphibians with compromised immunity to infectious diseases, such as the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), which could be particularly significant for populations intended for reintroduction. The development of treatments against Bd will need to consider a range of complexities regarding the microbial ecology of symbiotic bacterial communities on the skin of amphibians, particularly in the context host-microbe-environment interactions. The purpose of this research was to determine the impact of husbandry practices on symbiotic bacterial communities of frogs maintained at Chester Zoo and the University of Manchester. Specifically, the research aimed to:

1) investigate the effect of a carotenoid-rich diet on symbiotic bacterial communities of red-eyed tree frogs, and

2) assess the effects of varying ultraviolet light provision and calcium diets on growth, body condition and symbiotic bacterial communities of red-eyed tree frogs.

No effects of either UV treatment or calcium diet on growth or body condition of frogs were found. Specific dietary conditions (carotenoid availability) in captivity were found to alter the

symbiotic bacterial communities associated with the frogs' skin, whereas others (UV and calcium availability) have no effect although subsequent to the UV boost, frogs had a significantly greater fungal load in comparison to frogs that were not UV-boosted (Antwis et al., 2014a; 2014b). Thus, feeding and UV provision may influence the successful establishment of probiotics and affect the suitability of captive populations for reintroduction into the wild. In summary, host-microbe-environment interactions were identified pertinent to developing treatments for Bd and chytridiomycosis. At Chester Zoo, by gut-loading their cricket prey items, frogs are fed a carotenoid-enriched diet to enhance the skin color and promote species richness and abundance of cutaneous bacterial community. Boosting baseline UV light provision had no effect on growth, breeding success or symbiotic bacterial communities in these two species, and this costly addition to husbandry protocols has been stopped. Whether these bacterial changes increase susceptibility of amphibians to infectious disease is unknown and warrants further study.

Acknowledgments
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CONSIDERATIONS TO MAXIMIZE NUTRIENT SUPPLEMENTATION OF FEEDER INSECTS

Shannon E. Livingston, MSc*, Kathleen E. Sullivan, MS, and Eduardo V. Valdes, PhD

Animals, Science and the Environment, Disney's Animal Kingdom, Orlando, FL 32830 USA

Abstract

Providing a nutritionally appropriate diet to amphibians and other insectivores under human care has long proved challenging. The number of available insect species is limited and typically deficient in key nutrients such as calcium and vitamin A. Until more nutritionally balanced options are available, actions should be undertaken to improve the nutritional quality of feeder insects such as the domestic cricket (*Acheta domestica*), commonly used to feed amphibians and other insectivores. Common practices involve gut loading and dusting (Livingston et al., 2014; Oonincx and van der Poel, 2011). Gut loading utilizes a nutrient dense diet to feed the insect with hopes of improving its nutrient content through retention of the diet. Dusting involves coating the live insect in a powdered supplement prior to offering to the insectivore. Both methods of supplementation have been studied with regards to certain nutrients, specifically calcium and vitamin A/carotenoids, with inconsistent results reported in the literature (Livingston et al., 2014). Maximizing the efficacy of gut loading would require additional husbandry modifications for species like the domestic cricket. Utilization of recommendations involving temperature, supplementation regimen, water provisions, storage and preparation of vitamin supplements, particle size, and growth stage of the cricket, may to help maximize the effect of gut loading and supplementation of crickets (Attard, 2013; Livingston et al., 2014). Although considering factors such as the growth stage of crickets would help to optimize delivery, in practical systems this level of detail can be challenging, leading to a call for more streamlined and reliable products that deliver the most impact with the least labor intensity. There are a multitude of products available for gut loading and dusting, the reliability of which can vary significantly. One study previously reported that of four dry cricket gut loading diets tested, only three provided guaranteed calcium content and only two of those met their minimum guarantee (Finke et al., 2005). Some products are designed to provide only a specific nutrient or group of nutrients, and may be lacking other key nutrients. For example, insects raised to have enhanced vitamin content had calcium to phosphorus ratios ranging from 0.07-0.14 (unpublished), instead of the desired 1-2, and thus would still require gut loading or dusting to improve their overall nutritive value for the insectivore. When evaluating nutrient content and product efficacy, as is done at regularly at Disney's Animal Kingdom, often a gap exists between guaranteed analysis and reality. By evaluating current differences between product claims and analyzed values, we aim to demonstrate the need for scrutiny and consumer awareness to ensure delivery of proper nutrition to amphibians and other insectivores under human care. A multi-faceted approach to cricket care and feeding is necessary to ensure a nutritionally adequate diet for amphibians and other strict insectivores.

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NON-HEALING SUBCUTANEOUS HEMORRHAGE IN A COLONY OF VAMPIRE BATS (*DESMODUS ROTUNDUS*) DUE TO SUSPECTED VITAMIN C DEFICIENCY

Jennifer C. Hausmann, DVM[1]*, Jorden Manasse, BVMS[2], Howard Steinberg, VMD, PhD, Dipl ACVP[2], Victoria L. Clyde, DVM[1], and Roberta Wallace, DVM[1]

[1]Milwaukee County Zoo, Milwaukee, WI 53226 USA
[2]Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706 USA

Abstract

The Milwaukee County Zoo has housed vampire bats (*Desmodus rotundus*) since 1973. The bats are fed defibrinated cow's blood with a liquid pediatric multivitamin supplement. In November 2013, one bat developed a non-healing left wing hematoma. An August 2014 post-mortem examination revealed multifocal extensive necrohemorrhagic and suppurative ulcerative dermatitis with no underlying cause determined. From July to December 2014, five of nine bats in the colony developed similar hematomas along with gingival bleeding. Biopsies showed chronic histiocytic dermatitis with fibrosis and hemorrhage. One bat euthanized in December 2014 had a serum ascorbic acid level of 0.08 mg/dl and marked generalized subcutaneous hemorrhage. A nutritional imbalance was suspected, so a therapeutic trial was initiated in which two bats received defibrinated cow's blood supplemented with only oral vitamin C, 100 mg/kg p.o. × 3 days, and then 50 mg/kg p.o. daily. Two other bats received non-supplemented defibrinated cow's blood but were given vitamin K 3.3 mg/kg s.c. b.i.d. × 3 days, and then 3.3 mg/kg s.c. b.i.d. × 7 days. The bats supplemented with vitamin C improved, so all bats were then supplemented with vitamin C. All subcutaneous hemorrhages resolved within 10 days to 2 mo. Vitamin C is necessary for collagen synthesis, which is required for proper wound healing and capillary strength (Drouin, 2011). Many animals, including several bat species, cannot synthesize vitamin C and require a dietary source (Drouin, 2011). This is the first report of suspected vitamin C deficiency in a colony of vampire bats leading, to severe chronic subcutaneous hemorrhage.

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BODY WEIGHT CHANGES OF LEOPARD TORTOISES (*STIGMOCHELYS PARDALIS*) FED TWO ISOENERGETIC DIETS

Breanna P. Benson* and Mark S. Edwards, PhD

California Polytechnic State University, San Luis Obispo, CA 93047 USA

Abstact

Growth patterns of captive leopard tortoises (*Stigmochelys pardalis*) have been compared with those of free-ranging individuals (Hailey and Coulson, 1999; Rall, 1988; Ritz et al., 2010). Although useful comparative references for captive animal management, the lack of quantified food intake and opportunistic morphometric sampling associated with wild specimens limits their application. Average daily gain (ADG) of 17 female leopard tortoises born of the same clutch, was analyzed over two 12-mo periods: Period A was January through December 2012 (ages 2650-3015 days) and Period B was January through December 2014 (ages 3380-3745 days). Food offered supplied 50% of herbivorous reptile field metabolic rate (FMR, kJ ME/day) based on weekly body weight throughout both periods (Nagy et al., 1999). Daily food intake was quantified as the difference between food offered and orts remaining after 10 hr. On a dry matter basis, calculated metabolizable energy (ME; 12.93, 13.78 kJ/g), crude protein (CP; 13.7%, 16.3%), crude fat (CFat; 3.8%, 6.8%), and structural carbohydrates (crude fiber [CF]; 22.3%, 17.7%), differed for commercial diet blends consumed during Period A and B, respectively. The contributions (%) of CP and CF to consumed ME were consistent with previous recommendations (Donoghue and McKeown, 1999). NDF content was 51.4% and 40.7%, respectively. Absolute amount of ADG was significantly greater during Period A ($P < 0.05$) (Figure 1). Periods reported here represent ages beyond those for which growth has been previously described (Rall, 1988). Lower Period B growth rates may 1) be a result of reduced energy utilization associated with the nutrient profile consumed over that time and/or 2) coincide with a species typical, age-related decline associated with maturity.

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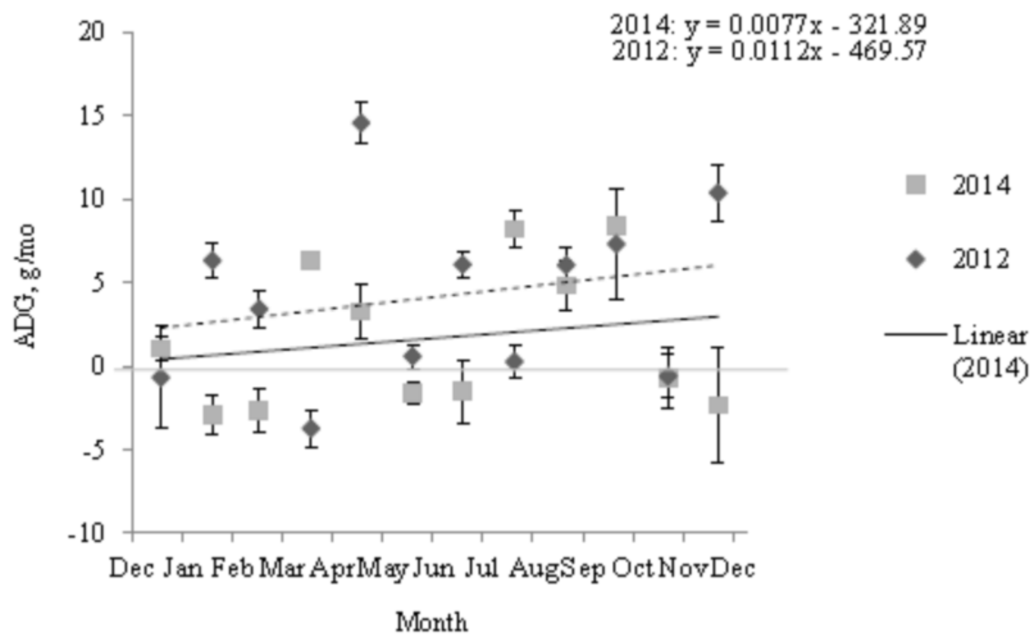

Figure 1. Mean average daily gain (ADG; ±SD) of female leopard tortoises (*Stigmochelys pardalis*; *n* = 17) fed isoenergetic diets over two 12-mo periods (Jan-Dec 2012 and Jan-Dec 2014).

EVALUATION OF THE NUTRITIONAL STATUS OF REHABILITATED GREEN SEA TURTLES (*CHELONIA MYDAS*) UTILIZING NUTRITIONAL MARKERS, STABLE ISOTOPES, AND METAGENOMICS

***Jennifer C.G. Bloodgood, MS*[1]**, Terry M. Norton, DVM, Dipl ACZM*[2]*, Lisa A. Hoopes, PhD*[3]*, and Sonia M. Hernandez, DVM, Dipl ACZM, PhD*[1,4]**

[1]*Daniel B. Warnell School of Forestry and Natural Resources, University of Georgia, Athens, GA 30602 USA*
[2]*Georgia Sea Turtle Center, Jekyll Island Authority, Jekyll Island, GA 31527 USA*
[3]*Georgia Aquarium, Atlanta, GA 30313 USA*
[4]*Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 USA*

Abstract
Green sea turtles (*Chelonia mydas*) are unique because hatchlings and pelagic juveniles are carnivorous, while later life history stages are primarily herbivorous. Dietary requirements at each life stage are poorly understood, making diet selection during rehabilitation of injured and sick animals challenging. Although turtles are typically transitioned to an herbivorous diet before release, food items high in animal protein (e.g., fish, shrimp, and squid) are often offered/consumed early in rehabilitation to combat poor appetite and emaciation. This may result in gastrointestinal pathologies and potential obesity.

To understand the impact of diet on health and recovery, nutritional parameters in green turtles undergoing rehabilitation at the Georgia Sea Turtle Center are being compared to those of healthy, free-ranging turtles captured during an ongoing monitoring project in St. Lucie County, Florida. Analyses include: (1) a suite of blood nutritional parameters (e.g., biochemical enzymes), (2) carbon and nitrogen stable isotopes in skin tissue, and (3) metagenomics of bacterial fecal flora. Rehabilitated turtles are monitored at admission, mid-rehab, and release. Preliminary analyses of blood nutritional parameters was completed on 13 rehabilitated turtles that were initially fed primarily carnivorous diets and then transitioned to primarily herbivorous diets pre-release. Turtles at the release time point had higher mean total protein ($P < 0.001$), mean triglyceride ($P < 0.001$), and mean ionized calcium ($P < 0.001$), and lower mean uric acid ($P < 0.05$) compared to the entry time point. Ultimately, information gained from this study will enable rehabilitation centers to make dietary modifications and develop gel-based diets that will enhance the recovery process for these endangered animals.

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EX SITU AMPHIBIAN NUTRITION: RECENT FINDINGS AND FUTURE DIRECTIONS

***Elizabeth A. Koutsos, MS, PhD*[1]* *and Eduardo Valdes, MS, PhD*[2]**

[1]*Mazuri Exotic Animal Nutrition, Shoreview, MN 55126 USA*
[2]*Department of Animal Health, Walt Disney World, Lake Buena Vista, FL 32830 USA*

Abstract

In 2013 a workshop was hosted to evaluate the current understanding of amphibian medicine and nutrition, in order to further our progress in establishment of successful breeding programs to promote amphibian conservation (Olea-Popelka et al., 2014). The Nutrition Working Group identified a number of challenges and opportunities for advancement in this field (Ferrie et al., 2014). First, an overarching theme of all Working Groups was the need for standardization of protocols and processes to allow for objective evaluation of nutritional and health status of amphibians. Second, an overview of current knowledge in amphibian nutrition was conducted. Finally, specific areas of concern in nutrition were highlighted for vitamin A status (determination of status, preventive and therapeutic options), multifactorial issues contributing to metabolic bone disease, the role of water quality in amphibian nutrition, establishment of appropriate research models to further define nutrient requirements of amphibians, and the need for evaluation of a wider range of wild-type diet items and enhancement of captive diet items.

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CHEETAH NUTRITION: RECENT ADVANCES AND REVISED SSP RECOMMENDATIONS

Ellen Dierenfeld[1]*, Katherine Kerr[2,3], Katherine Whitehouse-Tedd[4]

[1] Ellen S. Dierenfeld Nutrition Consulting, LLC, St. Louis, Missouri, USA
[2]University of Florida, Gainesville, Florida, USA
[3]Disney's Animal Kingdom, Lake Buena Vista, Florida, USA
[4] Cheetah Outreach, Somerset West, South Africa

Abstract
The SSP Cheetah Animal Care Manual was recently revised and updated, and is due for release by the end of 2015. As part of this process the Nutrition chapter was re-written in order to incorporate recent research findings and provide a more comprehensive overview of our current understanding. A review of the *in situ* diet of the free-ranging cheetahs demonstrated a preference for medium-sized ungulate prey, although smaller prey such as hares and large prey including kudus were also important dietary components according to various studies (Marker et al., 2003; Hayward et al., 2006). The prey preference of the wild cheetah is thought to reflect morphological adaptations, prey availability within cheetah range, as well as behavioral considerations for a species which is typically unsuccessful in defending prey against kleptoparasites. In order to best utilize findings from wild cheetah diets when evaluating or formulating diets for captive animals we compiled a table comparing the nutrient composition of the various natural prey species. A significant gap in the literature was subsequently identified whereby nutritional composition data was typically missing or only partially available (e.g. excluding micronutrients such as essential vitamins and minerals) for the majority of wild prey and even their domestic counterparts. For example, few data exist on the nutrient composition of whole ungulate carcasses; extrapolation of mineral data from Holstein calves suggested that trace minerals may be quite variable in particular, and the contribution of bone or non-meat carcass elements to nutrient profiles was unknown.

In terms of recent advances in our understanding of cheetah digestive function, the role of the gastrointestinal microbiota was highlighted. Researchers in cheetah (and other carnivore species) nutrition are increasingly including changes in the microbial community of the hindgut as a key parameter by which to assess dietary influence on animal health (Depauw et al., 2011, 2014b; Becker et al., 2014). Perturbations of the gut microbial populations and resultant fermentation activities can have important consequences for the overall health of the cheetah. Hind gut fermentation is highly influenced by diet, and in particular the fibrous (indigestible) component is considered important in this respect. Evidence for the beneficial effect of both plant and animal fibers in the fermentation patterns of captive cheetahs indicates that whole prey diets, or meat-diets supplemented with an appropriate plant fiber source, are necessary to promote gut health (Vester et al., 2008; Depauw et al., 2011; 2014; Kerr et al., 2013a). To this end, the use of pre- and probiotics was evaluated, and the potential pre-biotic activity of animal or plant fiber sources was considered worthy of further investigation (Koeppel et al., 2006. Vester et al., 2010; Depauw et al., 2011; 2012; de Godoy et al., 2013; Kerr et al., 2013a; 2013b). Whilst species-specific nutrient requirements are still unknown for the cheetah, known requirements for the domestic cat, as a model for the cheetah, were included for various life stages (weaning,

maintenance and reproduction; NRC, 2006). These values, alongside values reported for wild-type prey, provide an important benchmark against which to evaluate captive diets. Recent epidemiological evidence has revealed a geographical bias towards the use of commercially prepared diets in North America, compared to supplemented muscle meat and carcass provision outside of this continent (Whitehouse-Tedd et al., 2015). The majority of commercially prepared diets comprised ground horse or beef meat and the relative lack of fiber in these diets warrants a cautionary approach to their use as the sole source of nutrition for captive cheetahs. Furthermore, an epidemiological association was demonstrated between the feeding of horse meat on a regular basis (once a week or more) and an increased risk of gastritis (Whitehouse-Tedd et al., 2015).

A recommendation was made towards the inclusion of size-appropriate bones to improve dental health, encourage natural feeding behaviors, and provide supplementary animal fiber. However, the practice of providing animals with gnawing bones in the absence of a meal, i.e. on fasting days was discouraged. Moreover, the use of fasting days is no longer advised for cheetahs due to preliminary research indicating potentially negative consequences of fasting on animal behavior and fecal consistency. Instead, feeding enrichment, including randomized feeding schedules, should be prioritized over set fasting days (Quirke and O'Riordon, 2011).

Nutritional disorders are unfortunately still relatively frequently reported in captive cheetahs. Whilst the requirement for a balanced Ca:P ratio has been known for many decades, incidents of MBD have been reported as recently as 2011 (Bell et al., 2011). Additionally, copper deficiency and hypervitaminosis A were among a number of nutritional disorders still requiring particular attention in this species. Typically inappropriate supplement regimes were etiological in the nutritional disorders reported, including insufficient copper supplementation of poultry-based diets (Kaiser et al., 2014), or excessive provision of vitamin A (or liver).

Diet suitability and monitoring of nutritional status were highlighted as key elements to any nutrition program. Databases such as Zootrition® or Fauna® enable the chemical composition of most diets to be evaluated without the need for laboratory analyses, whilst standardized fecal consistency and body condition scoring systems are also available for this species (Dierenfeld et al., 2007; Whitehouse-Tedd et al., 2015). The signs of nutrient deficiencies or toxicities are well reported in the scientific literature and via the NAG and therefore widely available to zoological facilities. Combined, these monitoring tools provide ample opportunity for the monitoring of cheetah nutritional health to be incorporated into regular and routine veterinary examinations as well as daily keeper assessments. Likewise, protocols for the conduct of digestibility and feed intake can be found online, whilst the monitoring of serum biochemistry (Dierenfeld, 1993; Bechert et al., 2002; Depauw et al., 2012; Kaiser et al., 2014), as well as certain biomarkers of inflammation (Depauw et al., 2014a; 2014b) are available for those facilities interested in pursuing more comprehensive evaluations. However, it is imperative that the review of dietary suitability should include the services of a qualified animal nutritionist in order to ensure correct data interpretation.

Combining global research and feeding practices, including information from range habitats and integrated scientific disciplines, for any of our managed species provides a more comprehensive understanding and rapid advances towards optimized diets and nutritional management.

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UTILIZATION OF PORK AND PORK BY-PRODUCTS FOR NUTRITIONAL MANAGEMENT OF CAPTIVE EXOTIC FELIDS

Cayla Iske[1], Cheryl Morris[1,2], Kelly Kappen[2]

[1]Iowa State University, Ames, IA, United States.
[2]Omaha's Henry Doorly Zoo & Aquarium in Omaha, NE, United States.

Abstract

Currently beef and horse comprise the majority of raw meat diet formulations marketed by US companies manufacturing and supplying carnivore diets to zoological institutions. Pork-based diets have traditionally not been fed to managed exotic carnivores, primarily because of microbial and pathogenic concerns, and nutrient digestibility of pork has not been evaluated in captive exotic felids. Additionally, the pork industry currently sends many by-products to rendering that have potential use in raw carnivore diets. The overall objectives of this research were to evaluate a raw, pork-based diet for small and large captive exotic felids, including diet compositional analyses, digestibility, fecal scores, palatability, and microbial loads.

In our first aim, we demonstrated that a raw pork-based diet was highly digestible in large exotic felids by using four raw meat dietary treatments: one horse-based (Horse), two beef-based (B1, B2), and one pork-based diet (Pork). DM and CP apparent digestibilities were higher ($P < 0.05$) in cats fed Pork (87.97 and 95.74%) compared with cats fed Horse (83.59 and 92.71%) and B2 (85.60 and 93.14%). Apparent OM digestibility was higher ($P < 0.05$) in cats fed Pork (90.76%) than cats fed Horse (88.53%). Apparent fat digestibility values were high across all treatments but were higher ($P < 0.05$) in cats fed Pork (98.51%) compared with cats fed B1 (95.51%) or B2 (96.45%). Gross energy (GE) digestibility values were higher in cats fed Pork (92.38%) compared with B1 (90.21%). Average fecal scores were 2.30, 2.94, 3.42, and 3.54 (on a scale of 1 to 5) for Horse, Pork, B1 and B2, respectively; and were different between every treatment with the exception of B1 and B2 which were not statistically different. The pork-based diet was palatable and was selected by captive large exotic felids in 24 of 37 total (64.86%) observations of first approached and 23 out of 33 total (69.70%) observations for first tasted, compared to a raw beef-based diet.

In our second aim, we showed that a raw pork-based diet was highly digestible in small exotic felids by using raw horse (Horse), beef (Beef), beef/horse blend (Blend), and pork-based (Pork) dietary treatments. All diets were highly digestible, especially fat digestibility (98.58 to 99.73%) in which there were no statistical differences between diets. Digestibility of OM was higher ($P < 0.05$) when cats consumed the Blend diet (97.15%) compared to the Pork diet (93.10%). Fecal scores ranged from 1.55 to 2.63, with Beef (2.63) being statistically higher ($P < 0.05$) than Horse (1.55) and Pork (1.91). Additionally, microbial counts were shown to be highly variable in dietary treatments (E. Coli: 110 to 10,000 cfu/g; total coliforms: 150 to 28,000 cfu/g; yeast: 20 to 4,000 cfu/g; mold count: not detectable to 10 cfu/g; aerobic plate count: 23,000 to 26,000,000 cfu/g). Staphylococcus aureus was not detected in any of the diets. Salmonella was presumptive positive in the Pork and Blend diet, and was negative in the other three diets, but no signs of clinical illness were observed in cats fed any of the evaluated diets. Additionally, salmonella was not serotyped or quantified.

In conclusion, a pork-based raw meat diet is highly digestible and palatable to captive exotic felids and may be a valuable addition among dietary options for zoo managed carnivores. Additionally, microbial populations (aerobic plate counts) in commercial zoo raw meat diets are extremely variable. Therefore, reasonable standardized guidelines may need further consideration for these diets including the evaluation of pathogens not typically analyzed in quality control measurements such as Toxoplasma and Listeria. If large variations in aerobic plate counts do not change clinical outcomes, more specific and meaningful food safety analyses for zoo-managed carnivores may be warranted.

FEEDING STRATEGIES IN WILD CARNIVORES: PROGRESS REPORT OF A MODEL APPROACH

Annelies De Cuyper[1]*, Marcus Clauss [2], Myriam Hesta[1], and Geert P.J. Janssens[1]

[1]Laboratory of Animal Nutrition, Department of Nutrition, Genetics and Ethology, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19 9820 Merelbeke, Belgium
[2]Clinic for Zoo Animals, Exotic Pets and Wildlife, University of Zurich, Winterthurerstrasse 260 CH-8057 Zürich, Switzerland

Abstract
Feeding strategies among wild carnivores are still partly unexplained. Carnivore mass is a determining factor in the choice for a specific range in prey size (Carbone et al., 1999). According to Carbone et al. (2007), two dietary groups can be distinguished: small carnivores hunting on small prey (< 20 kg) and large carnivores hunting on large prey (> 20 kg). Prey size in his turn might influence feeding habit-associated factors such as meal size, feeding frequency and kill frequency but this has, hitherto, been left unexplained. A literature search was performed and yielded 690 potentially eligible studies of which already 194 (57 species) were extracted with data on feeding habit-associated factors. Moreover, a theoretical kill frequency model was developed based on carnivore weight, typical prey weight, maintenance energy requirements of mammals and metabolizable energy in small prey (Plantinga et al., 2007). So far, carnivore mass was positively related to absolute prey mass ($P < 0.001$). The kill frequency model showed that carnivore mass was negatively related to the theoretical kill frequency ($P < 0.001$), meaning that large carnivores will kill less frequently than smaller carnivores. This model approach might, once fully elaborated, give more insights into feeding strategies in wild carnivores with possible further applications for carnivores in captivity and domestic carnivores.

Introduction
In general, wild carnivores spend a high amount of energy hunting their prey. It is hypothesized that hunting energy expenditure becomes a determining factor for prey size in relation to carnivore size: smaller prey is easier to catch, but at a certain point, the energy cost per prey exceeds the energy gain in terms of nutritional value. In the literature, it has been suggested that wild carnivores switch from small- to large-prey-feeding at a body mass threshold of about 20 kg (Carbone et al., 1999; 2007). A relatively large prey size would logically imply a large relative meal size unless the larger prey has to be shared among so many individuals that meal size drops to levels of solitary predators of relatively small prey (Hornocker, 1967; Owen-Smith and Mills, 2008). Increasing meal size implies decreasing feeding frequency (i.e. increasing the time between meals), consequently decreasing hunting behavior (i.e. kill frequency) which is triggered by hunger (Hall and Bradshaw, 1998). This suggests that the choice for a specific range in prey size by carnivore species may have co-evolved with adaptations in meal size and kill frequency. Efforts have been made for different individual carnivore species to document their feeding habits but no broad literature survey is available, and the existing information is fairly scattered. Gathering this information could help to improve our understanding in the digestive physiology and satiety mechanisms not only in wild carnivores but also in carnivores in captivity and even domestic carnivores.

Materials and Methods

A literature review was performed from January to August 2014 using Web of Knowledge, PubMed and Google Scholar to identify potentially eligible studies reporting feeding habits of wild carnivores. The literature search yielded 690 potentially eligible studies of which already 194 (57 species) were confirmed eligible and extracted with data. Per carnivore species, data on prey species, prey mass (kg), most frequent prey (%), meal size (kg/day/carnivore), kill frequency (1 kill/x days) and pack size were extracted. Data were log transformed and linear curve fitting was performed on the relationship carnivore mass vs. mean prey mass of most common prey and a provisional regression equation was derived. Additionally, a theoretical kill frequency model was developed based on carnivore weight, typical prey weight, maintenance energy requirements of mammals and metabolizable energy in small prey (Plantinga et al., 2007).

$$\text{Total amount of prey (kg/carnivore/day)} = \frac{\text{MER (kJ/kg}^{0.75}\text{/day) x kg}^{0.75}\text{ carnivore}}{\text{ME prey (kJ/kg fresh weight)}}$$

$$\text{Theoretical kill frequency (kills/day)} = \frac{\text{Total amount of prey (kg/carnivore/day)}}{\text{Average prey weight (kg)}}$$

Data were log transformed and linear curve fitting was performed on the relationship carnivore mass vs. theoretical kill frequency and a provisional regression equation was derived. So far, pack size was not modeled in for both relationships. Meal size data turned out not usable for analysis and were therefore omitted from the database.

Results

Carnivores ranging in mass from 0.620 kg to 188 kg were included in the dataset. Carnivore mass was positively related to absolute prey mass ($P < 0.001$). Smaller carnivores (< 20 kg) typically hunt prey smaller then themselves, large carnivores (> 20 kg) hunt prey that are similar in weight or larger than themselves. Insectivorous carnivores (both large and small carnivores) showed prey masses of less than one gram. The kill frequency model showed that carnivore mass was negatively related to the theoretical kill frequency ($P < 0.001$), meaning that large carnivores will kill less frequently than smaller carnivores.

Discussion

Carnivore mass was positively related to prey mass as also shown by Carbone et al. (1999). The theoretical kill frequency decreased as predator mass increased which could be expected since a relative large prey implies a relative large meal, rendering long term satiation, which in turn leads to a low kill frequency (Hall and Bradshaw, 1998; Hornocker, 1967; Owen-Smith and Mills, 2008). However, caution is warranted when interpreting the results. Insectivorous carnivores show kill frequencies up to one kill/100 days indicating that the theoretical model does not apply to insectivorous carnivores. Therefore, insectivorous carnivores will be omitted, putting the focus on vertebrate prey feeders. The previous relationships might be influenced by the pack size of carnivores (i.e. solitary hunters vs group hunters). Therefore, inclusion of a pack size factor in both relationships is crucial. Moreover, the kill frequency model needs to be personalized per carnivore species in terms of MER values and energy contents of typical prey, taking feeding selectivity into account. Finally, data on kill frequency from literature are necessary to confirm the theoretical kill frequency model. Analyzing feeding strategies of wild carnivores will show how the choice for a specific range in prey size by carnivore species co-evolved with adaptations in meal size, kill frequency and even satiation mechanisms, rendering two possible strategy groups: the large carnivores showing a typical feast and famine style whereas smaller carnivores are typical frequent feeders. Unraveling these strategies in the wild might render possible applications for carnivores in captivity and even domestic carnivores.

Acknowledgements
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A COMPARATIVE NUTRIENT ANALYSIS OF FISH SPECIES CONSUMED BY MANAGED AND FREE-RANGING COMMON BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATES*) WITH RESPECT TO AMMONIUM URATE NEPHROLITHIASIS

Amanda J. Ardente, D.V.M.[1]*, Richard C. Hill, Vet.M.B., Ph.D.[2], Karen C. Scott, Ph.D.[2], Brian J. Vagt[2], Randall S. Wells, Ph.D.[3]

[1]University of Florida College of Veterinary Medicine Aquatic Animal Health, Gainesville, FL, 32608, U.S.A.
[2]University of Florida College of Veterinary Medicine Small Animal Clinical Sciences, Gainesville, FL 32608, U.S.A.
[3]Sarasota Dolphins Research Program, Chicago Zoological Society, c/o Mote Marine Laboratory, Sarasota, FL 34236, U.S.A.

Abstract
Ammonium urate nephroliths develop in common bottlenose dolphins (*Tursiops truncatus*) managed under human care, but do not occur in free-ranging dolphins (Smith et al., 2013). In mammals, urate urolith development has been attributed partly to the effect of diet on urine saturation and pH. Free-ranging and collection dolphins consume diets that differ in fish species variety, location, and fresh versus processed states. The proximate analysis and mineral content were measured in eight fresh frozen fish species (n=5) commonly consumed by free-ranging dolphins and seven stored frozen species commonly fed to collection dolphins. Metabolizable energy (ME) was calculated using Atwater factors. The dietary cation-anion difference (DCAD) was calculated as (Na^{+} + K^{+} + Ca^{2+} + Mg^{2+}) - (Cl^{-} + $P^{1.8-}$ + S^{2-};Frassetto et al., 1998). Nutrient concentrations relative to ME were compared among all fish species and between free-ranging and collection diet species. All nutrient concentrations differed (P < 0.0001) among all fish species. Concentrations of calcium, phosphorous, and DCAD were higher and chloride was lower in the free-ranging species (P < 0.05). The free-ranging species DCAD was positive (94 mEq/Mcal), whereas the collection species DCAD was negative (-70 mEq/Mcal). Thus, collection dolphins may have to excrete more anions, resulting in a more acidic urine. These nutrient differences may increase ammonia excretion in the urine and contribute to ammonium urate nephrolith formation in dolphins managed under human care.

Acknowledgements
We are grateful for the generous financial support of the United States Navy Marine Mammal Program, the National Marine Mammal Foundation, and SeaWorld Parks and Entertainment, Inc., without which this project would not have been possible. Additionally, we would like to thank SeaWorld Orlando and the U.S. Navy Marine Mammal Program, who worked together to provide the collection diet frozen species analyzed.

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A TARGETED METABOLOMICS ASSAY TO MEASURE PURINES IN THE DIET OF MANAGED AND FREE-RANGING COMMON BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATES*)

***Amanda J. Ardente, D.V.M.* [1]*, *Timothy J. Garrett, Ph.D.*[2], *Karen C. Scott, Ph.D.*[3], *Richard C. Hill, Vet.M.B., Ph.D.*[3]**

[1]University of Florida College of Veterinary Medicine Aquatic Animal Health, Gainesville, FL, 32608, U.S.A.
[2]University of Florida Southeast Center for Integrated Metabolomics Core Laboratory, Gainesville, FL, 32608, U.S.A.
[3]University of Florida College of Veterinary Medicine Small Animal Clinical Sciences, Gainesville, FL 32608, U.S.A.

Abstract
Ammonium urate nephrolithiasis occurs in common bottlenose dolphins (*Tursiops truncatus*) managed under human care but not in free-ranging dolphins (Smith et al., 2013). In mammals, purine-rich diets, such as the piscivorous diet of dolphins, can predispose to urate urolith formation (Osborne et al., 1995). The total purine content of food is measured commercially by summing the concentrations of four purine metabolites: adenine, guanine, hypoxanthine, and xanthine. Nevertheless, several other dietary purine metabolites can be converted into uric acid, and individual metabolite concentrations can vary with fish species, cold storage methods, and storage time (Clifford et al., 1976). A method using high-performance liquid chromatography with tandem mass spectrometry was developed to quantify the purine metabolites in frozen stored whole fish species [striped mullet (*Mugil cephalus*) and ladyfish (*Elops saurus*)] fed to collection dolphins and fresh frozen species [Atlantic herring (*Clupea harengus*) and west coast Loligo squid (*Loligo opalescens*)] consumed by free-ranging dolphins. The method accurately quantifies three additional metabolites: adenosine monophosphate, inosine monophosphate, inosine, and uric acid. The mean total purine content (n=5) measured using this assay, including these additional purine metabolites, was approximately twice that which would be quantified by the commercial assay. Additionally, mean inosine monophosphate concentrations were much greater on an energy basis in ladyfish when compared with the other species (656 vs. 2-18 μmol/Mcal ME; $p < 0.05$). Thus, this assay provides a more accurate determination of the total purine content of whole fish and reveals the extent to which individual purine metabolite concentrations vary among fish species.

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We are grateful for the generous financial support of the United States Navy Marine Mammal Program, the National Marine Mammal Foundation, and SeaWorld Parks and Entertainment, Inc., without which this project would not have been possible. Additionally, we would like to thank SeaWorld Orlando and the U.S. Navy Marine Mammal Program, who worked together to provide the managed diet frozen fish species analyzed. We also are thankful for the support of Dr. Randall S. Wells and the Chicago Zoological Society's Sarasota Dolphin Research Program for providing me with many opportunities to procure the free-ranging fish studied in this analysis.

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HYPOVITAMINOSIS A: INFLUENCE OF THREE DIETS OR TOPICAL TREATMENT ON HEPTATIC, ADIPOSE AND PLASMA RETINOID CONCENTRATIONS AND PRESENCE OF SQUAMOUS METAPLASIA IN MISSISSIPPI GOPHER FROGS (*RANA CAPITO SERVOSA*)

Christina Ploog[1]*, Robin Clunston[2], Cheryl Morris[1,3], Cayla Iske[3], William Blanner[2] and Allen Pessier[4]

[1]Animal Health, Omaha's Henry Doorly Zoo and Aquarium, Omaha, NE
[2]Division of Preventative Medicine and Nutrition, Columbia University Medical Center, NY, NY.
[3]Comparative Nutrition, Department of Animal Science, Iowa State University, Ames, IA
[4]Wildlife Disease Laboratories Institute for Conservation and Research, San Diego Zoo Global, San Diego, CA

Abstract

Hypovitaminosis A has been linked to health concerns in amphibians causing squamous metaplasia of the tongue, bladder, kidneys and other organs. Juvenile Mississippi Gopher Frogs (*Rana capito servosa)* at Omaha's Henry Doorly Zoo and Aquarium (n = 32) were randomly assigned to one of four vitamin A treatment groups including three dietary feeder cricket treatments and one topical vitamin A treatment. Dietary treatments included the zoo's standard protocol for feeder insects (gut loaded and dusted) (Control), equal portions of kale, carrots and sweet potato (Carotenoid) or a diet consisting of ground Mazuri® Aquatic Turtle food (77%), fish oil (12%) and spirulina (11%) similar to the diet used by Li et al. (2009; Enhanced). Enhanced and Carotenoid fed crickets were also dusted twice weekly with MinerAll® and once weekly with Herptivite® similar to control crickets. The topical treatment provided 50 IU vitamin A per 10 grams of body weight, every other day (Topical). Control crickets were fed to frogs receiving the Topical treatment. Animals were housed in groups of four for 60 days and euthanized at the conclusion of the study for tissue collection. Liver, blood and adipose tissues were submitted for vitamin A analyses. Tongue and kidney tissues were evaluated histopathologically. Hepatic retinol concentrations were highest ($P < 0.05$) for frogs fed the Carotenoid treatment (5.50 nmol/g) compared to the Enhanced treatment (3.50 nmol/g). Hepatic retinyl esters were highest ($P < 0.05$) for the Carotenoid treatment group (73.16 nmol/g) compared to Enhanced and Control groups (39.32 and 37.24 nmol/g, respectively). Hepatic beta-carotene and adipose retinoid concentrations were not different among groups. Plasma retinol concentrations were highest ($P < 0.05$) for the Topical treatment (0.151 μm) compared with Enhanced (0.127 μm). No additional differences were detected. No squamous metaplasia was found in any tissues. Dietary treatments did include dusting supplementation of crickets prior to feeding; therefore, further evaluation may be needed because all treatments appeared to provide adequate vitamin A to prevent squamous metaplasia for the length of study. Additionally, further evaluation of toxicity should be considered, though no signs of toxicity were noted. Topical vitamin A provided similar effectiveness as diets, although those animals also received Control crickets. Increases in hepatic retinoid concentrations were only detected with the Carotenoid diet, indicating that amphibians likely convert beta-carotene to retinol and store it in their liver. Plasma concentrations were conserved across groups indicating that circulating concentrations of retinoids are likely not accurate measures of vitamin A status.

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A REVIEW OF THE NUTRIENT CONTENT OF COMMERCIAL FEEDER INSECTS AND STRATEGIES FOR INCREASING THEIR NUTRIENT CONTENT

Mark D Finke PhD

Mark Finke LLC, Rio Verde, AZ 85263

Abstract

Insects are an important source of nutrients for a wide variety of captive insectivores including many reptiles and amphibians. Nutrient analysis of many of the more common commercially available feeder insects has now been published. These data are reviewed and discussed in light of the fact that unlike many free ranging animals, insectivores in captivity are fed a limited number of different species of insects and therefore may be more prone to nutritional deficiencies. Nutrient deficiencies reported in the literature for various species of captive insectivores include calcium deficiency, vitamin A deficiency and thiamine deficiency. Additionally nutrients where intake is likely to be low based on chemical analysis of commercially available insects include vitamin E, vitamin B_{12}, carotenoids and omega-3 fatty acids. Feeding management strategies for optimizing nutrient intake of captive insectivores fed commercially available feeder insects are presented. In addition, a review of the various methods for enhancing nutrient content and the advantages and disadvantages of each will be reviewed. These methods include dusting the insect with a nutrient rich powder, gut-loading the insect with a nutrient dense diet and changing the feed of the insect during growth to optimize key nutrients.

NUTRIENT COMPOSITION OF THE MILK OF THE GIANT ANTEATER (*MYRMECOPHAGA TRIDACTYLA*).

Michael L. Power

Smithsonian Conservation Biology Institute, Nutrition Laboratory, Washington DC

Abstract
Little information exists about milk composition in obligate insectivores or in members of the mammalian superorder Xenarthra. A total of 37 milk samples collected from 3 lactating giant anteaters (*Myrmecophaga tridactyla)* were assayed for proximate nutrient content at the Nutrition Laboratory of the Smithsonian Conservation Biology Institute using standard methods developed at the Nutrition Laboratory. Water comprised 88.7 ± 2.1% of the milk (mean ± SD). Sugar and fat content were moderately low (3.2 ± 0.6% and 1.0 ± 0.5%, respectively). Crude protein was the highest solid constituent at 5.6 ± 1.2%. Milk gross energy content (GE) was 0.55 ± 0.11 kcal/g.

Giant anteater milk appears to be an example of milk with moderately low sugar and fat and high protein content. On an energy basis giant anteater milk contains 103 ± 9 mg of protein per kcal, the highest value for protein on an energy basis of milk from any species so far assayed at the Nutrition Laboratory. Protein energy accounted for 60% of GE. If all the protein, sugar and fat in the milk were metabolized into energy (ME), protein would contribute 52% of ME.

Not all the milk protein will be metabolized, of course. Some will be used to build tissue. But the high proportion of potential metabolizable energy in giant anteater milk from protein strongly suggests that milk protein is an important energy source for the neonates. This is also probably true for adults; a diet of ants is likely high in protein, moderate at best in fat, and low in carbohydrate (as well as high in dirt). Catabolism of protein into glucose would be predicted to be important for adult giant anteaters.

A reliance on milk protein to provide neonates with metabolizable energy may be a feature of Xenarthran lactation. Nine-banded armadillo milk is also high in protein (87 ± 12 mg/kcal) and low in sugar (2.7 ± 0.5%), though it is not low in fat (5.2 ± 2.2%). Adult armadillo diet also is high in protein, and probably low in fat and carbohydrate. There are no data on sloth milk as yet; but sloths have a diet high in mature leaves, which typically are high in protein, and low in fat and simple sugars. The extant Xenarthrans might share a milk composition that reflects milk protein as an important neonatal energy source. Further research is needed to determine the extent to which this hypothesis reflects evolutionary reality.

CASE STUDY: THE ANOREXIC ANTEATER

Heidi Bissell

Busch Gardens Tampa, 3605 E. Bougainvillea Dr., Tampa, FL 33604 USA

A DIFFERENT ANGLE ON GEOMETRICAL ANALYSIS OF DIETS: DEVELOPING A TOOL FOR USING GEOMETRY TO COMMUNICATE NUTRITION TO LAYPEOPLE

Heidi Bissell

Busch Gardens Tampa, 3605 E. Bougainvillea, Tampa, FL 33612

Abstract

Geometric analysis provides an intuitive and powerful tool to examine the diet and feeding behavior of animals. Because of its visual nature, it may also be a powerful tool to assist in communications with laypeople, such as clients or zoo staff members, who are often intimidated by a more numerical analysis. A new tool, a ternary graph using color for a fourth axis, allows the visualization of diets on a simple graph. By comparing a proposed diet to defined regions for that species, a layperson can quickly see whether or not a proposed diet fits within a target range. By making "live" adjustments to the diet together, the nutritionist and client can converge on a diet that is balanced nutritionally but also encompasses the training, enrichment and social needs of the animal.

Introduction

Geometric analysis, a technique developed by Raubenheimer and Simpson (1997, 1999) provides an intuitive and powerful tool to see how diets converge on nutritional targets, to identify nutritional priorities of animals, and examine how different diet ingredients contribute to the overall diet and feeding behavior of animals. One use of geometric analysis that has not been as thoroughly explored is using nutritional geometry to communicate with laypeople, such as clients or zoo staff members. Over 60% of the British population is considered "innumerate" or has a strong negative emotional reaction to numbers (Coben et al. 2003), and the US lags behind England in many mathematical surveys. In my experience, showing some clients a traditional diet analysis (i.e., results from a nutrition lab or a diet composition calculated on a spreadsheet) can be very intimidating. Human nutritionists have spent a great deal of effort developing ways to increase nutritional literacy in human populations. They've developed widely used tools such as standard serving sizes, Food Pyramids, uniform nutritional labeling, the MyPlate tools, diabetic exchange tables, point systems, and others. What these tools share is their focus on visual depictions and simple metrics that laypeople can understand. The goal of this paper is to present one possible way that something similar might be accomplished in a zoo setting.

The goal of a nutritional consultation in a zoo or similar setting is typically to find a diet that meets the animal's estimated nutrient requirements while simultaneously meeting training, enrichment and social goals for the animals. A positive, collaborative approach involves working side-by-side with the zookeeper or manager to find a workable compromise between the foods they would like to feed and those that meet the nutrient requirements of an animal. Unfortunately, this often involves balancing diets in front of them and exposing them to complex tables of numbers. While some staff are receptive to this and interested to learn more, many others have more negative reactions, and this can hinder the collaborative diet design process.

With a small amount of orientation, I've found that using a modified geometric framework graph provides a non-threatening, visual way to develop diets cooperatively with non-nutritionists.

The original tool developed by Simpson and Raubenheimer (1995; 1999) depicted two nutrients (a third nutrient could be evaluated on a diagonal axis with training; Fig 1). While this provided a valuable new tool to the field of nutritional ecology, these graphs are not always easily interpreted by laypeople. Human nutritionists have begun using ternary graphs (triangle plots), with the three axes being the proportion of calories from protein, fat, and carbohydrates, to communicate individual food or diet composition (Fig 2). Different popular human diet plans aim for different regions of this triangle (Fig 2b; http://nutritiondata.self.com).

In the realm of animal nutrition, Hewson-Hughes used ternary plots very effectively in his work identifying the nutritional targets of domestic cats. These graphs provide a visually compelling story of cats' dietary selection goals when consuming dry food (pink triangle) and canned food (blue triangle) diets (Fig 3; Hewson-Hughes et al. 2011).

Although these plots work well for carnivores, these ternary plots have a flaw that becomes apparent when using them for non-human, non-carnivore diets – they group all carbohydrates together. A diet containing mostly sugar will appear in the same area as one containing mostly fiber, despite the fact that these have very different physiological implications. A constant challenge in zoo diets for many animals is keeping the non-structural carbohydrate (NSC) proportion (primarily sugars and starch) low, while maintaining adequately high levels of structural carbohydrates (SC, cellulose, hemicellulose, etc.). To visually display this would require a fourth axis (protein, fat, NSC, SC), which would render a ternary graph challenging to read.

The goal of this project was to develop a simple, visually effective way to communicate macronutrient composition of a diet, including separating structural and non-structural carbohydrates. In addition, this tool should allow "live" comparisons of a proposed diet with a recommended target range.

Methods

Fourth axis

A ternary plot using protein, fat, and carbohydrate has shown itself in both human and animal nutrition to be an effective communication tool. To address the need for a fourth dimension (separating carbohydrates into SC and NSC), I used *color* as a fourth axis, with a scale that ranges from green (at least 80% of the carbohydrate calories are from non-structural carbohydrates) through yellow (at least 40%) to red (less than 20% of the carbohydrate calories are from non-structural carbohydrates).

Caloric content

ME: The caloric content of the whole diet was calculated using published metabolizable energy (ME) values of the individual ingredients. When different ME values were available for different taxa (i.e., foregut fermenters, hindgut fermenters, primates, birds, etc.), the value deemed most relevant for the particular species was used. If only one ME value was available, it was used regardless of animal taxa. Because ME data were missing entirely for some diet ingredients, diets for which less than 95% of the dry matter had published ME values were excluded. 6 diets out of 146 were excluded, leaving 140 diets in the analysis.

Protein and Fat ME: The caloric contribution of each macronutrient in the diet was estimated using Atwater's values of 9 kcal/g of fat and 4 kcal/g of protein.

Total Carbohydrate, By Difference: The total amount of carbohydrates was estimated using the USDA method of "carbohydrates, by difference (TC)": 100- (protein + fat + ash + water).

SC ME: To determine the calories from NSC vs. SC, several estimations had to be made. To estimate SC, the maximum value of NDF, ADF, CF and TDF was used. Obviously these four analyses are not directly comparable to one another. The calories from SC were estimated as 1.9 kcal/g * SC.

NSC ME: Very few ingredients had starch or sugar values available. Therefore, NSC was also determined by difference as: TC – SC. NSC was estimated to contain 4 kcal/g.

NSC:SC: The proportion of NSC was determined as NSC (kcal)/Total Carbohydrate (kcal).

Target ranges

To determine the target ranges for different groups of animals, a sample of 80 unique diets were selected (a single diet shared by many animals was just used once) that had been in use for at least a year with minimal changes and no known metabolic issues. Although it is not known whether these diets are "ideal", they appear to be working well. Like all zoo diets, they have been adjusted over time to meet the various needs of our animals and are currently our best estimate as to an optimal diet at this time.

Each diet was graphed on a ternary plot by diet type. Species were divided into the following diet type groups based on nutritional ecology and taxonomy: carnivores (consumers of whole vertebrate prey including mammals, birds, and reptiles), insectivores (many xenarthrans, bushbabies, smaller amphibians and reptiles), browsers (black rhinos, giraffe, gorillas, sloths, etc.), grazers (zebras, white rhinos, cattle, and hippos), frugivores, omnivores (mostly small mammals and rodents), and avian generalists (mostly small granivorous birds).

For "live" interactions with keepers, an Excel spreadsheet is used with a ternary plot generated by code by Fernando Cinquegrani (http://www.prodomosua.eu/ppage02.html). For the fourth axis, color was used to describe the proportion of SC to NSC. Red was used for diets containing more than 80% of the carbohydrate calories from NSC, orange for diets between 60 and 79.9%, yellow for diets between 40 and 59.9%, lime green for diets between 20 and 39.9%, and dark green for diets that were extremely low in carbohydrates, such as carnivore diets.

Within each manually classified group, the NSC/SC proportion was averaged (**Table**) and the entire group was assigned to a single color band based on the average.

Results

The regions graphed can be viewed in an interactive demo at:
https://zoonut.shinyapps.io/Triangle3/

Discussion & Conclusion

The development of the colored regions has been very helpful both for analyzing diets and working with the keepers. When analyzing diets, oddities where a diet falls outside of the expected range can be quickly noted and examined. For example, flamingos were classified as carnivores, but fall quite far outside the other carnivorous bird diets. Potentially, we might reconsider the composition of our flamingo diets, or reclassify flamingos as omnivores or avian generalists rather than as carnivores.

When I meet with keepers, we can simply work with the familiar diet ingredient amounts and the ternary graph. I begin by adding in all of the keepers' requests for training and enrichment items, and we then adjust the diet together until their diet (as depicted on the graph) is the correct color and within the target range. This can be done without staff ever encountering a single number. In my experience, if we generate a diet that (a) contains feeds typical for that species (b) that falls within the target region and (c) is the correct color, then balancing micronutrients requires very few additional adjustments. The impact of adding some high-sugar training or enrichment items becomes quickly apparent, and educational conversations about how to meet training goals while not unbalancing a diet can take place more easily.

In short, this tool offers a visually appealing technique that appeals to many nutrition clients and makes balancing diets collaboratively easier. It has the potential to allow us to evaluate diets against a new scale. Although this tool is based off one institution's current zoo diets, this technique could be expanded to include wild diets or diets from multiple institutions.

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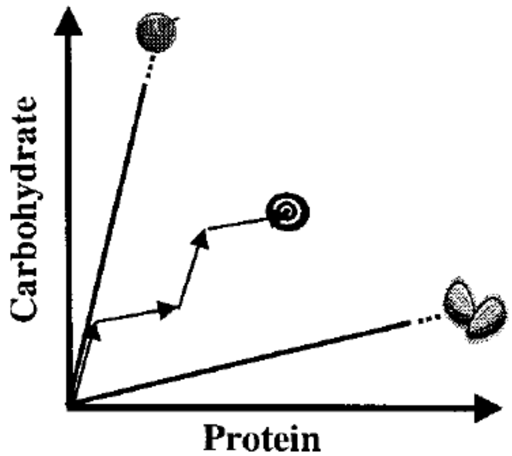

Figure 1. An example of geometric analysis where an insect might select from among two foods, neither of which is ideal, in order to reach a nutritional target (Raubenheimer and Simpson 1999).

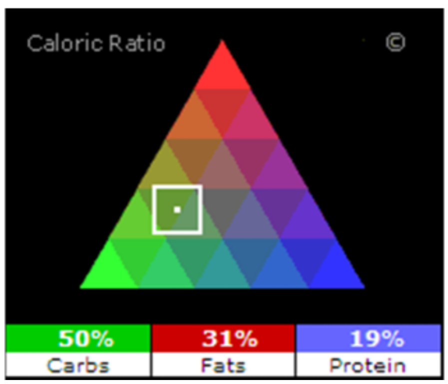

Figure 2a. The use of a ternary diagram in human nutrition.

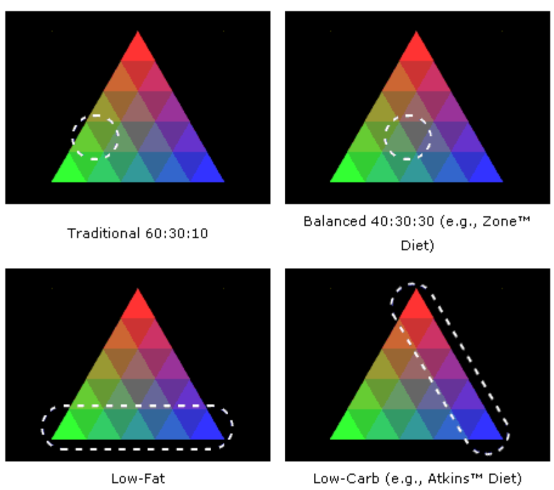

Figure 2b. Human diet plans as graphed on a ternary graph. (http://nutritiondata.self.com/help/analysis-help#cp-pyramid)

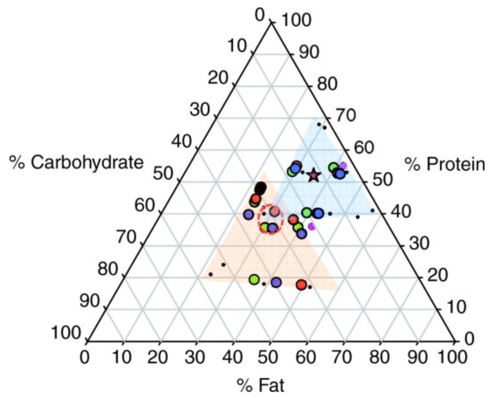

Figure 3. Dietary selection for particular nutrient targets by domestic cats. When cats were offered 3 dry diets at the apices of the red triangle, they selected a target within the red circle. When they were offered diets at the apices of the blue triangle, they selected a mixture within the red star (Hewson-Hughes et al. 2011).

Table 1. Ratio of non-structural carbohydrate (NSC) to structural carbohydrate (SC)

DietType	Color	Average	SD
Carnivore	Dark green	18%	19%
Grazer	Light green	21%	9%
Browser	Yellow	59%	9%
Omnivore	Orange	67%	18%
Insectivore	Orange	75%	9%
Frugivore	Red	80%	12%
Avian	Red	94%	6%

WHAT GOES AROUND, COMES AROUND. THE CASE FOR TRANSFAUNATION

Mike Maslanka, MS[1]* and Amanda Guthrie, DVM, Dipl. ACZM[2]

[1]Department of Nutrition Science, Smithsonian Conservation Biology Institute, Washington, DC, USA
[2]Virginia Zoo, Norfolk, VA, USA

ANALYSIS OF NUTRIENTS, MOISTURE LOSS, AND VITAMIN STABILITY IN PRIMATE BROWSE HARVESTED AT THE SAN DIEGO ZOO

Rhianne M. Maxwell, BS[1,2]* and Michael L. Schlegel, PhD, PAS, Dipl ACAS[2]

[1]Animal Science Department, California Polytechnic State University, San Luis Obispo, CA 93407 USA.
[2]Nutritional Services Department, San Diego Zoo Global, San Diego, CA 92112-0551 USA.

Abstract
The objective of this study was to obtain nutrient profiles along with insight on rates of moisture and vitamin loss in browse harvested at San Diego Zoo Global (SDZG). Ten species of browse commonly offered to colobines were manually separated into leaf and bark components for analysis at a commercial laboratory. Findings indicate that a variety of browse species and dietary items are essential to meet nutrient requirements. *Morus alba* was utilized for the water retention and vitamin stability trials. Browse that was presented upright or upside down without water for 24 h lost 12-14% of the original leaf moisture compared to browse presented upright in water for 24 h, which only lost 4% of the original leaf moisture (difference numerical, not statistically significant, $P > 0.05$). Large variance in the data suggests that the study evaluating water loss should be repeated. Refrigerating the *M. alba* browse at 4°C maintained the α-tocopherol concentration (270 µg/g DM) for 172 h post-harvest, and β-carotene concentration (225 µg/g DM) was maintained for 100 h before decreasing 23% after 172 h post-harvest (difference numerical, not statistically significant, $P > 0.05$). Alpha-tocopherol and β-carotene remained relatively stable; subsequent studies on vitamin stability could incorporate longer storage time of *M. alba*.

Introduction
Three projects performed during the summer of 2014 included: browse components and nutrient assessment, browse water retention, and vitamin stability in browse offered to primates. Nutrient profiles of browse offer information to improve dietary formulation. Browse composition is especially important to consider when it constitutes a significant portion of an animal's diet. In order to collect data regarding browse offered to leaf-eating primates, 10 species of browse plants grown at San Diego Zoo Global (SDZG) were analyzed. One of these species, white mulberry (*Morus alba*), was used to study water retention and vitamin stability.

Mulberry is a deciduous plant that grows quickly in various climates and is utilized as feed for insects and animals (Kandylis et al., 2009). To explore the effect that presentation methods may have on rate of moisture loss, *M. alba* was subjected to three different treatments and monitored for water retention. Moisture retention over 24 h was examined to improve presentation for increased consumption. Wild colobines have demonstrated the ability to select leaves based on moisture content (Mckey et al., 1981; Mowry et al., 1996), which may affect palatability and receptivity among primates (Laska et al., 2000).

Additionally, mulberry leaves are valued for nutritive and antioxidant components, including α-tocopherol and β-carotene (Yen et al., 1996). In response to the installation of a cooler dedicated to primate browse at the San Diego Zoo (SDZ), the stability of α-tocopherol, and β-carotene concentrations in *M. alba* leaves was determined over 172 h (7 d). Alpha-tocopherol is the most bioavailable form of vitamin E, and β-carotene is the precursor to vitamin A (Robbins et al., 1994).

Materials and Methods

Nutrient Profiling

The browse species analyzed included orchid tree (*Bauhinia galpinii*), magenta lily pilly (*Eugenia peniculata*), weeping fig (*Ficus benjamina*), Chinese banyan (*Ficus microcarpa*), crossberry (*Grewia occidentalis*), Hawaiian hibiscus (*Hibiscus rosa sinensis*), white mulberry (*Morus alba*), cape honeysuckle (*Tecomaria capensis*), rosewood (*Tipuana tipu)*, and garden nasturtium (*Tropaeolum majus*). All samples were harvested around 0500 and stored in a large, outdoor cooler (4°C) until 0900 retrieval. Branches (46 cm) were stored by species in bundles of 10 branches and kept dry. Upon retrieval, the browse was transported to the Nutrition Laboratory where whole branch bundle weights were obtained.

Each portion of the plant was manually removed. Leaves and flowers were removed by the stem base, and bark was stripped from the wood. All components of the deconstructed browse were dried separately for a minimum of 48 h in a 55°C forced-air drying oven to gravimetrically determine moisture content. Dried specimens were ground through a 2.0 mm screen (Thomas Wiley® Mill, Thomas Scientific, Swedesboro, NJ 08085). Plant proportions were calculated based on weight.

Ground bark and leaf subsamples weighing 65 g or greater were submitted for proximate analysis (Dairy One, Ithaca, New York 14850). Nutrients analyzed included crude protein, crude fat, detergent fiber, starch, total ash, and select trace minerals. Due to limited sample size, only *T. majus* flowers were analyzed. An increased branch sample size would yield more floral material

Moisture Loss

M. alba branches were harvested from SDZG for use in the moisture loss and vitamin stability study. Harvest and delivery of the bundles to the primate browse cooler occurred between 0500 and 0900. The bundles were retrieved at approximately 0900.

M. alba, commonly offered to primates at the SDZ, was evaluated for water retention when presented in three different methods, using a control and positive control. The methods of presentations were control, initial leaf DM; upright; upside down; upright in water; and a positive control, stored upright in a 4°C cooler for 24 h. Fifteen branches of *M. alba* (46 cm) were received each morning for four consecutive days (four replicates). Bundles of three branches were randomly assigned to one of the presentation treatments: control, deconstructed upon retrieval; upright with no water source; upside down with no water source; upright in an enclosed PVC pipe containing water; and upright in a cooler with no water source. The control was processed upon retrieval at 0900 by manually removing the leaf from the stem at the base of the node. The remaining branch bundles were secured onto a fence in correspondence to the assigned treatment using zip ties. All bundles were secured within 61 cm (24 in) of one another with moderate exposure to both sunlight and shade. Each bundle was removed from treatment conditions and processed after 24 h of exposure.

Dry matter was obtained gravimetrically after a minimum of 48 h in a 55°C forced-air drying oven. Changes in moisture content by treatment were analyzed using single factor ANOVA (MS Excel®) with means separated by Tukey-Kramer test (Gill, 1987). Means were considered significantly different when $P < 0.05$.

Vitamin Stability

Branches (91 cm) were harvested between 0500 and 0900, and stored without water in a 4°C cooler until scheduled processing date. On three separate days (replicates) the stability study was started by randomly assigning 25 branches to 5 sampling times. Five branches were sampled at 4, 28, 52, 100, and 172 h post-harvest.

Leaves were manually separated from branches and stored in a -20°C freezer until samples were transported under dry ice to be freeze dried (Flexi-Dry MP™, SP Industries, Stone Ridge, NY 12484; DuoSeal® Vacuum Pump 1402, Welch-Ilmvac, Niles, IL 60714). The maximum length of time from collection and storage to freeze drying was 30 d. Leaves were dried for a minimum of 48 h, or until a constant weight was obtained. Freeze-drying was selected over air drying due to reported antioxidant activity sensitivity to temperature (Katsube et al., 2009).

Dried leaves were ground (Thomas Wiley® Mill, Thomas Scientific, Swedesboro, NJ 08085) through a 2.0 mm screen. Samples were sent to a commercial laboratory (Michigan State University DCPAH, East Lansing, MI 48910-8104) for α-tocopherol and β-carotene analysis. Changes in vitamin concentration were analyzed using single factor ANOVA (MS Excel®) with means separated by Tukey-Kramer test (Gill, 1987). Means were considered significantly different when $P < 0.05$.

Results

Nutrient Profiling

Browse branches (76.7 g) contained 54.5% leaves, 15.8% bark, 28.6% wood, and 1.3% flowers (Table 1). *T. tipu* was not included in the averages since total leaf weights were not recorded and *T. majus* was not included because distinct vines were not separated. In general, the browse leaves had greater protein (15.4%) and fat (4.4%) and lower ADF (20.2%), NDF (34.6%) and lignin (6.9%) than bark (protein, 7.2%; fat, 2.3%; ADF, 38.2%; NDF, 49.2%; lignin, 9.0%) (Table 2). The exceptions are the leaves from *T. tipu* that had greater NDF than the bark and the leaves of *T. capensis* that had greater lignin than bark.

Calcium content of *Ficus* spp. leaves and bark were greater (3.1-4.8%) than the other browse species (Table 3). *H. rosa sinensis* leaves had the highest P content (0.8%). *G. occidentalis*, *H.rosa sinensis*, *M. alba*, *T. capensis* and *T. majus* had leaves, bark or both that had K content greater than 2.4%. *H. rosa sinensis* (leaves) and *T. majus* (leaves and vines) were high in Na (> 1.2%). The S content of the *T. majus* components were over 1% and the *T. majus* leaves had the highest Cl content (1.7%). *F. microcarpa* (leaves and bark) and H. *rosa sinensis* (leaves) had greater Fe content (> 477 ppm) than the other browse species. *T. majus* leaves had more than twice the Zn content (137 ppm) compared to the other browse species leaves. *T. capensis* had the greatest Mn content (67 ppm).

Moisture Loss

There were no statistical differences ($P > 0.05$) observed between browse-presentation treatment groups (Table 4). Browse that was presented upright or upside down for 24 h lost 12-14% of the original leaf moisture compared to the 4% moisture loss in leaves from browse that was presented upright in water for 24 h. The large variance suggests that the study should be repeated.

Vitamin Stability

The concentration of α-tocopherol (270 μg/g DM) in *M. alba* leaves did not decrease ($P > 0.05$) following storage up to 172 h in a 4 °C cooler (Table 5). Although not statistically significant (P>0.10), β-carotene concentration (225 μg/g DM) was stable for 100 h post-harvest in the cooler and then decreased 23% after 172 h post-harvest.

Discussion

Nutrient Profiling

Food items, which are infrequently analyzed, may be unaccounted for in diet formulation or inaccurately represented in current diets. The species included in this study were chosen due to a combination of limited published data and to obtain current analyses for browse species grown at SDZG and fed to colobines.

Leaves in addition to bark were analyzed to account for foraging behavior observed in SDZG primates, in which the bark is often stripped and consumed. Because woody portions of these plants are seldom ingested, wood was not analyzed. Seasonal comparison of the browse could capture nutrient variation throughout the year.

Comparing the nonhuman primate requirements (NRC, 2003) to the data in Table 2 and 3, no single species of browse would solely meet the requirements. By providing a varied diet along with a commercial primate biscuit, nutrient requirements can be met.

While these analyses provide specific data regarding browse grown at SDZG, this information can be extrapolated for use in other facilities. Data representing the nutrient content of these species can aide in diet formulation.

Moisture Loss

Colobines, such as Asian langurs, rely on fruits and vegetation as a primary source of water, rarely opting for free water (Harris, 1970). This would cause speculation that primates may choose to select foods containing higher water content. Moisture content of browse may also affect palatability among primates (Jildmalm et al., 2008). Leaves containing higher levels of moisture are associated with younger plants, which are characterized by an abundance of nutrients and lower fiber in comparison to a mature plant (Mowry et al., 1996). Wild colobus monkeys show selectivity for young plant leaves, only consuming mature plant leaves when resources are scarce (Mckey et al., 1981; Mowry et al., 1996). Additionally, wild ring-tailed lemurs displayed preference for foliage with higher water content, leading to speculation that primates may favor plant material based on moisture content (Mertl-Millhollen et al., 2003). Several studies, however, have demonstrated no or negative correlation for primate preference among fruits and leaves with high water content, possibly due to less nutrient density (Jildmalm et al., 2008; Laska et al., 2000). While this preference was not examined in this study, identifying the type of browse presentation that will improve utilization will help reduce food waste at SDZ. A follow-up trial repeating the procedure to reduce variance in combination with examining preference could be explored.

Vitamin Stability

Motivation for this study stems from findings in which α-tocopherol was stable in *Eucalyptus sideroxylon* leaves for 141 h post-harvest when refrigerated (Desai and Schlegel, 2011). The only loss of nutrients in *E. sideroxylon* with refrigeration was a loss of non-fiber carbohydrates (NFC). The current study suggests that during a 172 h period, no significant change was detectable in the measured antioxidant activity of *M. alba* leaves. A study with a longer cooler storage time would be required to follow the trend of decreased α-tocopherol or β-carotene concentration beyond 172 h (7 d). One study supports that no significant change in antioxidant activity occurs in refrigerated mulberry leaf extract until 60 days of storage (Arabshahi-Delouee and Urooj, 2007). Nonhuman primates require 8,000 IU vitamin A/kg DM (14.4 µg β-carotene/g) and 100 mg/kg (100 µg/g) vitamin E (NRC 2003). Sampled *M. alba* leaves contain adequate concentrations of α-tocopherol and β-carotene up to 172 h post harvest. Mulberry stems were not examined in this study, but can be a good source of antioxidants that could be considered for future work (Syvacy and Sokmen, 2004). Additionally, a non-refrigerated negative control could be used to demonstrate the benefits of refrigeration.

Findings emphasize that diets including browse should be varied to achieve nutrient requirements. *M. alba* presentation methods tested did not demonstrate significant differences of water loss, but the resulting large variance suggests that the study should be repeated. During a 172 h period, α-tocopherol and β-carotene concentration in *M. alba* leaves remained stable.

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Table 1. Primate browse component weights per branch and the calculated percentage of total branch weight (as-fed basis).

	Leaves		Bark		Wood		Flower	
Species	Wt per branch, g	% of branch weight	Wt per branch, g	% of branch weight	Wt per branch, g	% of branch weight	Wt per branch, g	% of branch weight
Bauhinia galpinii	47.7	45.8	15.3	14.7	40.9	39.3	0.2	0.2
Eugenia peniculata	79.7	58.2	9.9	7.3	47.1	34.4	0.1	0.1
Ficus benjamina	122.3	55.1	27.8	12.5	71.8	32.4	0.1	0.03
Ficus microcarpa	152.0	50.8	40.1	13.4	104.8	35.0	2.4	0.8
Grewia occidentalis	40.3	53.6	14.4	19.1	17.4	23.1	3.1	4.2
Hibiscus rosa sinensis	43.6	41.4	26.2	24.9	33.6	31.9	2.0	1.9
Morus alba	82.9	63.0	18.6	14.1	30.0	22.8	NP[1]	NP
Tecomaria capensis	45.3	68.4	13.3	20.0	6.6	9.9	1.1	1.6
Tipuana tipu	DNR[2]	DNR	258.3	DNR	469.7	DNR	0.4	DNR
Tropaeolum majus	29.3	16.5	142.5[3]	80.1	NP	NP	6.1	3.4

[1]Plant component not present.
[2]Data not recorded.
[3]Refers to vines.

Table 2. Nutrient composition of ten species of primate browse.

Species	Part	DM, %[1]	Crude Protein, %[1]	ADF, %[1]	NDF, %[1]	Lignin, %[1]	Crude Fat, %[1]	NFC[1,2] %	ESC[1,3] %
Nonhuman Primates[4]	-	NR[5]	15-22	5-15	10-30	NR	NR	NR	NR
Bauhinia galpinii	Leaves	46.9	15.3	16.3	24.2	4.6	2.9	50.9	10.4
	Bark	52.5	5.9	38.2	45.5	7.4	1.8	39.4	8.2
Eugenia peniculata	Leaves	28.6	10.1	19.3	25.4	6.1	4.8	55.8	6.6
	Bark	31.7	5.6	30.2	38.1	9.2	2.5	46.2	10.3
Ficus benjamina	Leaves	40.4	10.6	28.2	40.8	10.3	4.4	29.7	9.2
	Bark	39.3	6.3	49.5	60.7	13.8	2.3	17.1	4.0
Ficus microcarpa	Leaves	37.7	8.0	24.7	34.1	8.2	4.3	34.4	5.8
	Bark	43.1	3.8	30.1	37.2	9.1	2.6	40.6	9.3
Grewia occidentalis	Leaves	35.0	18.6	17.6	42.0	4.2	3.2	26.4	6.4
	Bark	38.5	8.0	39.0	66.0	6.9	1.4	15.8	6.6
Hibiscus rosa sinensis	Leaves	27.8	18.3	16.2	40.9	4.8	6.8	19.4	4.9
	Bark	27.8	7.0	51.6	65.0	6.4	1.6	15.1	4.8
Morus alba	Leaves	32.1	17.9	13.3	24.0	2.8	5.2	40.7	10.4
	Bark	30.7	8.8	34.3	41.3	7.0	4.2	37.6	7.2
Tecomaria capensis	Leaves	34.3	19.3	22.7	37.9	12.1	5.2	29.9	8.4
	Bark	36.7	8.7	36.0	49.1	9.0	2.0	33.2	11.5
Tipuana tipu	Leaves	35.1[6]	20.9	23.7	42.2	9.0	3.0	26.4	9.0
	Bark	43.9	10.5	34.8	39.7	12.1	2.1	40.3	10.5
Tropaeolum majus	Leaves	15.6	29.4	10.8	21.4	2.5	9.2	25.3	7.7
	Vines	7.7	15.5	32.7	38.1	5.1	2.5	24.3	11.1
	Flowers	14.4	26.0	11.4	20.1	2.3	7.7	36.9	11.9

[1]All nutrients, except for DM, reported on a dry matter basis. [2]Non-fiber carbohydrates calculated as 100% - (CP% + NDF% + Fat% + Ash%). [3]Ethanol soluble carbohydrates (simple sugars). [4]Published nonhuman primate requirements (NRC 2003). [5]No requirement established. [6]In the original dataset, the dry matter content could not be calculated due to missing data. The leaf DM listed is from unpublished data (San Diego Zoo Global).

Table 3. Macromineral and micromineral content (DM basis) of ten species of primate browse.

Species	Part	Ash, %	Ca, %	P, %	Mg, %	K, %	Na, %	S, %	Cl, %	Fe, ppm	Zn, ppm	Cu, ppm	Mn, ppm	Mo, ppm
Nonhuman Primates[1]	-	NR[2]	0.8	0.6	0.1	0.4	0.2	NR	0.2	100	100	20	20	NR
Bauhinia galpinii	Leaves	6.7	1.5	0.3	0.2	1.0	0.02	0.2	0.2	171.0	41.0	8.0	34.0	0.6
	Bark	7.4	1.7	0.5	0.1	1.4	0.01	0.1	0.4	63.0	59.0	7.0	18.0	1.2
Eugenia peniculata	Leaves	3.9	0.4	0.1	0.2	0.8	0.30	0.2	0.1	201.0	14.0	7.0	25.0	0.3
	Bark	7.6	1.4	0.1	0.4	1.2	0.31	0.1	0.2	99.0	13.0	10.0	18.0	0.0
Ficus benjamina	Leaves	14.6	3.4	0.1	0.3	1.4	0.03	0.2	0.1	157.0	16.0	5.0	29.0	0.2
	Bark	13.5	3.1	0.2	0.2	1.5	0.03	0.1	0.1	131.0	14.0	11.0	15.0	3.0
Ficus microcarpa	Leaves	19.1	4.8	0.1	0.5	1.5	0.10	0.2	0.3	569.0	31.0	7.0	36.0	0.2
	Bark	15.9	4.2	0.3	0.2	1.2	0.05	0.1	0.4	602.0	55.0	10.0	20.0	1.1
Grewia occidentalis	Leaves	9.8	1.7	0.3	0.3	2.4	0.02	0.3	0.7	195.0	68.0	15.0	46.0	1.0
	Bark	8.9	1.2	0.2	0.3	2.4	0.03	0.2	0.8	76.0	40.0	10.0	23.0	0.7
Hibiscus rosa sinensis	Leaves	14.6	2.2	0.8	0.5	1.3	1.35	0.7	0.5	477.0	63.0	12.0	39.0	0.8
	Bark	11.3	1.5	0.5	0.2	2.8	0.54	0.3	0.9	62.0	27.0	13.0	16.0	0.1
Morus alba	Leaves	12.2	1.9	0.6	0.4	2.4	0.01	0.2	0.3	209.0	27.0	5.0	36.0	2.8
	Bark	8.2	1.3	0.3	0.3	1.8	0.01	0.1	0.1	70.0	19.0	4.0	30.0	0.9
Tecomaria capensis	Leaves	7.7	1.1	0.3	0.3	1.3	0.54	0.2	0.8	212.0	36.0	9.0	67.0	1.5
	Bark	7.1	0.5	0.2	0.2	2.3	0.29	0.1	1.0	116.0	46.0	23.0	33.0	0.9
Tipuana tipu	Leaves	7.5	1.6	0.2	0.5	1.0	0.03	0.2	0.6	259.0	37.0	11.0	30.0	1.3
	Bark	7.5	1.9	0.2	0.3	1.0	0.02	0.2	0.4	147.0	48.0	13.0	12.0	1.4
Tropaeolum majus	Leaves	14.7	1.9	0.5	0.6	2.2	1.20	1.1	1.7	278.0	137.0	10.0	28.0	1.5
	Vines	19.5	1.5	0.4	0.5	4.8	1.52	1.1	0.8	121.0	63.0	6.0	6.0	1.1
	Flowers	9.2	0.4	0.7	0.3	3.1	0.15	1.0	0.3	129.0	80.0	10.0	18.0	0.0

[1]Published nonhuman primate requirements (NRC 2003).
[2]No requirement established.

Table 4. Moisture content of *Morus alba* leaves 24 h after browse presentation treatment.

	Treatment					
	Control	Upright	Upside down	Upright in water	Refrigerated	SEM[1]
Moisture, % as-fed	69.08	59.15	60.89	66.24	66.98	3.29

[1]Standard error of the means.

Table 5. Alpha-tocopherol and β-carotene concentration stability of refrigerated *Morus alba* leaves following harvest and refrigeration up to 172 h.

Vitamin	Hours post-harvest					
(DM basis)	4[1]	28	52	100	172	SEM[2]
α-tocopherol (μg/g)	251.0	272.67	286.6	262.7	276.1	33.83
β-carotene (μg/g)	230.9	214.9	231.2	224.8	176.7	19.71

[1]Initial nutrient concentration post-harvest prior to refrigeration.
[2]Standard error of the means.

BIRTH WEIGHTS AND GROWTH RATES OF GIRAFFE AND OKAPI AT DISNEY'S ANIMAL KINGDOM

Shannon Livingston[1]*, Kathleen Sullivan[1], Katherine Kerr[1,2], and Eduardo Valdes[1-4]

[1]Department of Animal Health, Disney's Animal Kingdom, Lake Buena Vista, FL, USA
[2]Department of Animal Sciences, University of Florida, Gainesville, FL, USA
[3]University of Guelph, Ontario, Canada
[4]University of Central Florida, Orlando, FL 32816 USA

Abstract

There is a lack of information available regarding the average birth weights and growth rates of many exotic animal species. This information can prove useful when evaluating the health of new born animals in a zoological setting. Within 24 to 48 hours of birth, giraffe (*Giraffa camelopardis*) and okapi (*Okapia johnstoni*) at Disney's Animal Kingdom are weighed and health is assessed as part of the regular neonate exam. Body weights are gathered as available to monitor growth. The birth weights of 19 giraffe and 5 okapi are reported within as are the growth curves of 21 giraffe and 5 okapi. The average birth weight of giraffe at Disney's Animal Kingdom (DAK) from 1998 through 2015 is 64.64 kg +/- 7.41 kg (n=19). The average birth weight of male giraffe at DAK is 65.83 +/- 7.34 kg (*n*=11) and female giraffe is 63.0 +/- 7.68 kg (*n*=8). By one year of age, the average weight of giraffe at DAK is 382 kg +/- 28.83 kg (*n*=17). The average birth weight of okapi at DAK is 16.0 +/- 0.64 kg (*n*=5). This is lower than the average birth weight of 19.6 kg reported by the Brookfield Zoo (Reason, 1991). By one year of age, the average weight of okapi at DAK is 175.75 +/- 3.18 kg (*n*=5). Growth curves for male giraffe through the first year are displayed in Figure 1 and those for female giraffe are shown in Figure 2. Growth curves for okapi are shown in Figure 3. It is hoped that this information may prove useful when assessing the health and growth of other giraffe and okapi in zoological settings.

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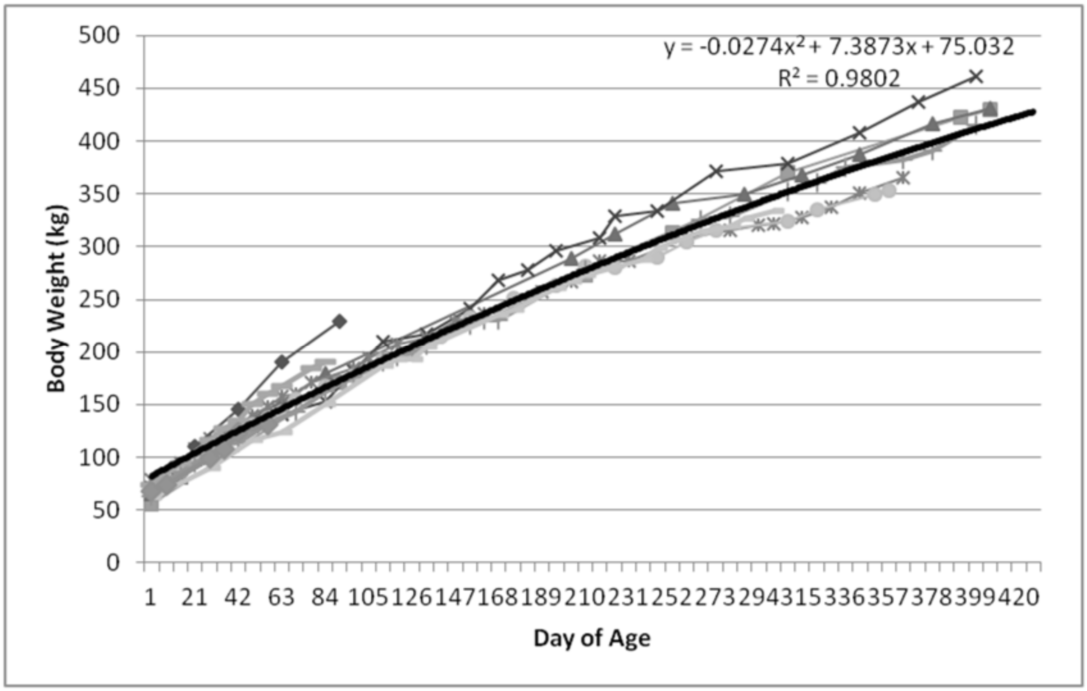

Figure 1. Growth of male giraffe through 1 year of age at Disney's Animal Kingdom

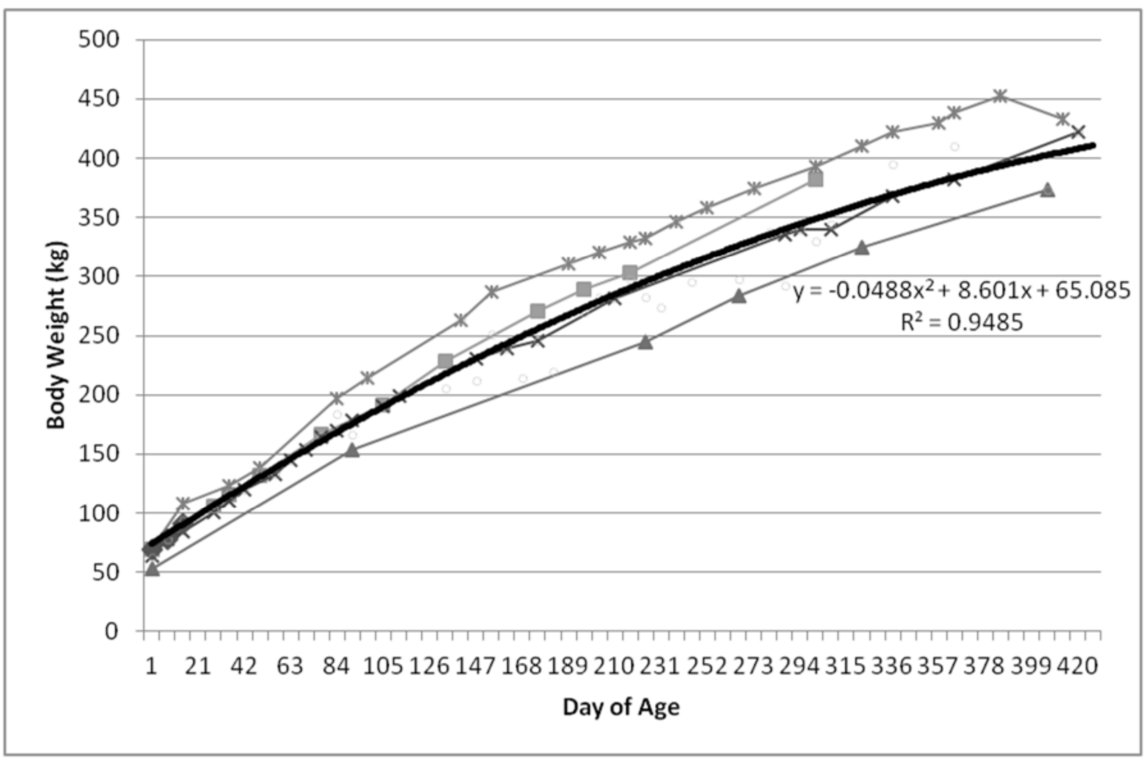

Figure 2. Growth of female giraffe through 1 year of age at Disney's Animal Kingdom

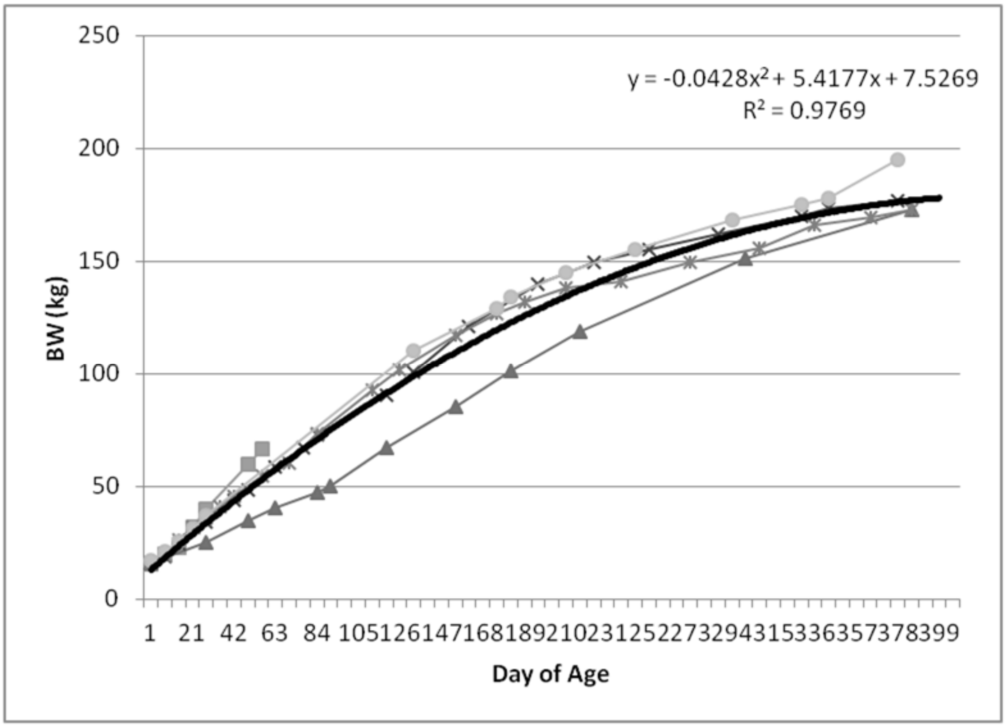

Figure 3. Growth curves of Okapi through year 1 at Disney's Animal Kingdom

CALIBRATION DEVELOPMENT FOR RAPID ASSESSMENT OF FISH SPECIES FOR DOLPHINS IN HUMAN CARE USING NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

Scott Williams[1], Kathleen Sullivan[1, 2], Shannon Livingston[1], Katherine Kerr[1, 2], and Eduardo V. Valdes[1-4]*

[1]Disney's Animal Kingdom, Lake Buena Vista, FL 32830 USA
[2]University of Florida, Gainesville, FL 32611 USA
[3]University of Guelph, Ontario, Canada
[4]University of Central Florida, Orlando, FL 32816 USA

Abstract
Near infrared reflectance spectroscopy (NIRS) is a fast, accurate, and cost effective method for analyzing organic compounds. Dolphins as a species are highly sensitive to changes in the energy and nutrient contents of their diets. We analyzed monthly samples (n=148) of capelin, herring, and squid for dry matter, crude protein and crude fat. The dried and ground samples were returned to our lab and analyzed via NIRS for collection of spectral data. Calibrations were developed using the wet laboratory analysis as reference values to develop prediction equations. We were able to produce strong equations with low standard error of cross validation (SECV) = 1.29 to 1.90; r^2= 0.90 to 0.98. These indicate good predictive power of our equation and will allow accurate assessment of the nutrient content of fish.

Introduction
Dolphins held under human care are sensitive to slight fluctuations in dietary energy. Earlier research from our laboratory found high variability of nutrient content in the fish species that compose a majority of dolphin's diets at Disney (Sullivan et. al., 2013). Regular analysis of the main dietary components allows nutritionists to adjust for these dietary fluctuations and maintain the dolphins within a target weight range. We aim to expand our Near Infrared Reflectance Spectroscopy (NIRS) program researching its utilization as a quick, accurate, and cost effective method for regularly assessing these fish species as both dried (Sullivan et. al., 2008) and test the ability to analyze fresh samples.

Materials and Methods
Monthly samples (n = 148) of capelin, herring, and squid were collected by the marine mammal team at the Seas and sent to our laboratory and also to Dairy One Forage Laboratory for analysis of dry matter (DM), crude protein (CP), and crude fat. Wet chemistry data were utilized as reference values for subsequent NIRS calibration development and cross validation. The dried and ground samples were returned to our laboratory, scanned by NIRS (SpectraStar 2500X-RTW System with InfoStar Software v. 3.11.1, Unity Scientific, Brookfield, CT), and reflectance spectra were collected over a range of 680 to 2500nm with 1nm wavelength increments. Calibration development was achieved by treating the full spectra with partial least squares. Cross validation of the prediction equation was achieved by dividing the calibration set into independent groups. Each group was pulled out of the calibration set, and tested by predicting their nutrient values against their laboratory value. This tested the predictive accuracy of the equation.

Results and Discussion

When the spectra for the dried fish samples were modeled via principal component analysis (PCA), the results indicated a balanced population that could be analyzed as a robust, mixed species prediction (Figure 1). When analyzed as a mixed group, the nutrient prediction equations for dried fish were very strong with low standard error of prediction (SE) = 0.86 to 1.86; r^2= 0.96 to 0.99 and low standard error of cross validation (SECV) = 1.29 to 1.90; r^2= 0.90 to 0.98. These results indicate a strong predictive power of our equation and will allow our laboratory to accurately assess the nutrient content of fish. In future research, to improve the efficiency of sample analysis, our laboratory will be assessing the ability of NIRS to predict blended, whole fresh fish samples to more easily assess the nutrient profiles presented to our dolphin population.

Acknowledgements

Thank you to the Marine Mammal team at the Seas for all of their work sampling for this project.

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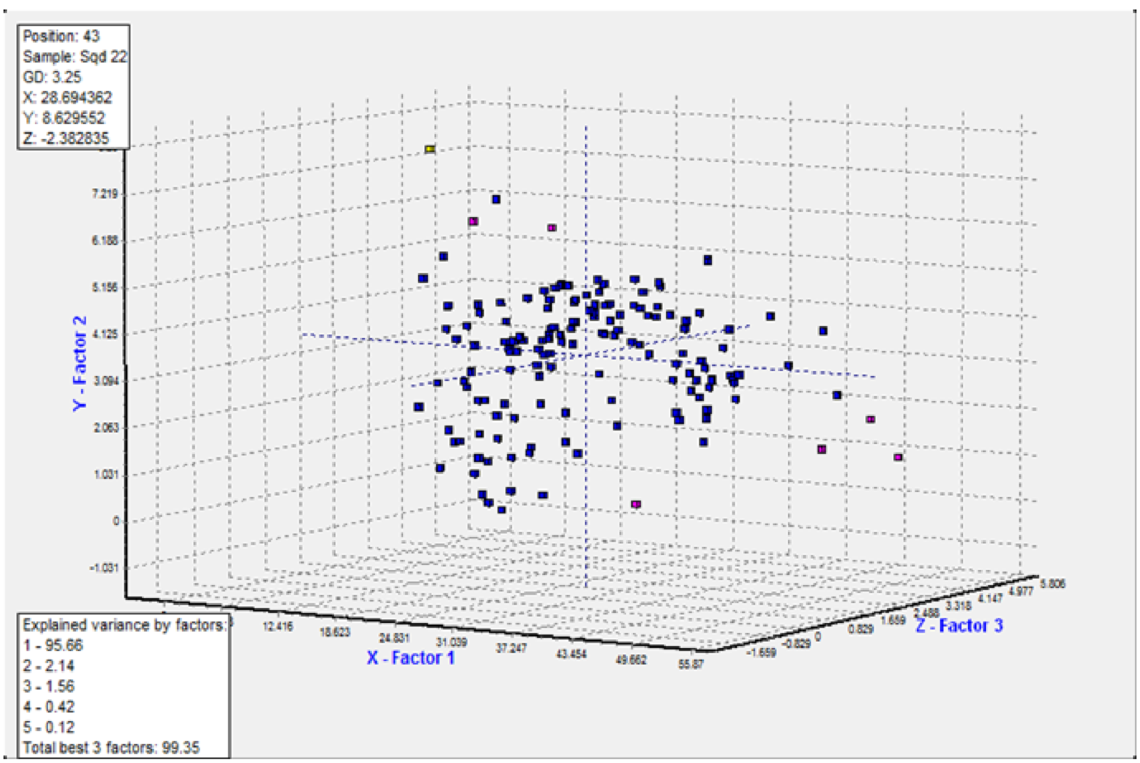

Figure 1. Principal component analysis of dried fish spectra, mixed Species.

COMMERCIAL AQUATIC DIETS AND WATER QUALITY

Katherine Kerr[1,2], Matthew Dawson[1], Kathleen Sullivan[1,2], Amber Thomas[1], Shannon Livingston1, Andy Stamper[1], Charles Staples[2], and Eduardo V. Valdes[1 - 4]*

[1]Disney's Animal Kingdom, Lake Buena Vista, FL 32830 USA
[2]University of Florida, Gainesville, FL 32611 USA
[3]University of Guelph, Ontario, Canada
[4]University of Central Florida, Orlando, FL 32816 USA

Abstract

Water quality is an important limitation of diet choice and feed amounts in ornamental aquaria. Advanced life support system technology (a.k.a. water treatment) allow aquaria to maintain proper water quality; however, addition of diet items to the water is regarded as a key component impacting water quality maintenance via the load presented to life support systems. Herein, we evaluated nutrient composition of three commercial diets and ran preliminary tests to compare their potential impact on water quality parameters.

We utilized three commercial diets (Mazuri, St. Louis, MO; Table 1): herbivore fish pellet diet (5E4R Aqua Herbi-Blend Pellet); carnivore fish pellet diet (5E4S Aqua Carni-Blend Pellet); and aquatic gel diet (5AB0 MTLS Aquatic Gel). Diets were immersed in deionized water (1 g dry matter / 100 mL; Table 2) for 3 hours, and water quality parameters were measured. A replicated cross-over design was utilized in two tanks (each diet tested 3 times per tank). Briefly, 8 g diet dry matter was weighed out and placed in a 1 mm sieve. The sieve was slowly immersed into an aquarium filled with 800 mL of deionized water. After 3 hours, the sieve was pulled out of the aquarium with all diet pieces and allowed to drain completely. Dried weight (dried at 105 C for 24-h) of the remaining pieces was recorded as percent of dry matter retained. Total ammonia nitrogen was measured via colorimetric assay according to the salicylate method (Method 8155, HACH, Loveland, CO) and read on a benchtop spectrophotometer (DR 6000, HACH, Loveland, CO). We measured pH prior to introducing diet to the water, and after diets had been immersed for 3 hours. Results are presented in Table 3.

The pH did not differ ($P > 0.10$) from zero, nor was there any significant differences ($P > 0.10$) between dietary treatment on pH at 3 hours (data not shown), or change in pH. These data indicate that no significant bacterial digestion of the diets occurred during the 3-h time frame. Aquatic gel had lower stability (i.e., 2% more DM was solubilized into the water after 3 hours). Additionally, the concentration of total ammonia as N was approximately 3 to 4 times higher in water exposed to aquatic gel compared to water exposed to the pelleted feed. This result is not surprising given the higher solubilization of the gel diet combined with the higher N load when compared to the pelleted diets. Concentration of N in these diets is lower than a majority of animal prey diet items commonly utilized in aquatic diets (75 ± 15% CP; Sullivan et al., 2015).

Fish diets can be high in protein (> 50% DM) which introduces a high load of nitrogen into the system. Therefore, in closed systems, it is important to feed nutritionally balanced diets (all necessary calories, vitamins, minerals, etc.) while minimizing the total load on the life support system. Commercially produced complete diets, including some pellets and gel diets, provide the opportunity for aquarists to feed a homogenous ration that provides the necessary nutrients uniformly throughout. Further experiments of commercial fish diets on water quality parameters, in aquarium housed fish are needed.

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Sullivan K, S Livingston, K Kerr, S Williams, and E Valdes. 2015. Common aquatic ingredient nutrient analyses: Balancing practical feeding with long term aquatic health. *Proceedings of the Nutrition Advisory Group to the AZA.*

Table 1. Nutrient composition of three commercial aquatic fish diets.

Item	Carnivore Pellet	Aquatic Gel	Herbivore Pellet
Dry Matter, %	92.3	22.6	94.2
Ash, % DM	13.3	11.3	10.8
Crude Protein, % DM	54.0	64.0	39.2
Crude Fat, % DM	10.0	16.7	9.6
Neutral Detergent Fiber, % DM	6.8	20.9	23.6
Acid Detergent Fiber, % DM	3.5	3.9	14.1
Starch, % DM	9.6	0.3	6.8
Simple Sugars, % DM	3.2	3.1	3.3
GE, kcal/g	4.844	5.364	4.721

Table 2. Total nutrients introduced to each 800 mL system.

Item	Carnivore Pellet	Aquatic Gel	Herbivore Pellet
As-Is, g	8.7	35.4	8.5
Dry Matter, g	8.0	8.0	8.0
Crude Protein, g	4.3	5.1	3.1

Table 3. Parameters after immersion of commercial diets (8 g dry matter) in distilled water (800 mL) for 3 hours.

Item	Carnivore Pellet	Aquatic Gel	Herbivore Pellet	SEM	P-Value
Water					
pH Change	0.246	0.018	-0.504	0.336	0.306
Nitrogen, Ammonia, mg/L	0.47^{b}	1.34^{a}	0.35^{b}	0.06	0.000
Diet					
Dry Matter Retained, %	83.2^{a}	81.0^{b}	83.3^{a}	0.29	0.000

COMMON AQUATIC INGREDIENT NUTRIENT ANALYSES: BALANCING PRACTICAL FEEDING WITH LONG TERM AQUATIC HEALTH

Kathleen Sullivan[1,2]*, Shannon Livingston[1], Katherine Kerr[1,2], Scott Williams[1] , Eduardo V. Valdes[1-4]

[1]Disney's Animal Kingdom, Lake Buena Vista, FL 32830 USA.
[2]University of Florida, Gainesville, FL 32611 USA.
[3]University of Guelph, Ontario, Canada.
[4]University of Central Florida, Orlando, FL 32816 USA.

Abstract

Optimizing dietary formulations for aquatic species is one of the most challenging aspects of nutrition for exotics. Whether designing diets for elasmobranches, fish in a multi-species aquarium, or marine mammals, understanding the nutrients provided in the food is essential. Ideally, designing aquatic diets utilizes both known body weights of the target species, and analyzed nutrient contents of diet items. Repercussions of overfeeding in an aquatic system can lead to water quality issues, and ultimately poorly understood compromised health in the occupants. While aquatic diets historically have been designed primarily based on energy needs, vitamins and mineral content should also be considered, especially for long term health.

Working with the aquarists at EPCOT, Disney's Animal Kingdom nutrition team conducts regular quality control sampling and compositional analysis to monitor nutrients in a variety of ingredients used for aquatic species (Table 1). We utilize Dairy One laboratories for proximate and trace mineral analysis, as well as our own laboratory for gross energy by bomb calorimetry.

For obesity sensitive species such as the bottlenose dolphin, turtles and black-blotched ray, diet adjustments based on caloric and dietary item intake paired with corresponding body weight data, body condition scoring, and blood draws are actively ongoing. The practical priority of aquarists is often feeding of energy, especially in target fed animals such as turtles and rays, where individual preferences on feed type may change over time. There are marked differences in nutrient content when dietary items are altered (decreased, increased, or removed). Changes in preference leading to changes in consumption will impact health; therefore interaction between teams is essential. The nutritionist provides an understanding of ingredient composition and supplement needs based on dietary ingredient consumption by the animals. For example, dietary calcium concentrations would decrease with use of de-shelled vs. shelled shrimp, de-penned vs. penned squid, or capelin with heads and tails removed vs. whole capelin. Also to be considered is the need for vitamin supplementation (primarily vitamin E), as almost 100% of the aquatic items sampled below are kept frozen and contain very low amounts of vitamin E once thawed (Crissey and Spencer, 1998; DAK quality control database). While much continues to be elucidated on understanding normals for aquatic species, in terms of serum, water, and requirements, knowing exactly what is provided through daily nutrition is a comparably simple piece of the puzzle to track. Information on daily ingredient amounts and corresponding nutrient information will continue to add to the understanding of the effect of diet on long term aquatic animal health.

Acknowledgements
The authors would like to thank the Seas team for samples and for working with us to maximize health through nutrition.

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Table 1. Nutrient Composition on Dry Matter basis of Feeds used in aquatic species diets

	DM	CP	Crude Fat	Ca	P	Mg	Fe	Zn	Cu	Mn	Mo	S	Se	Co	Gross Energy
Unit	%	%	%	%	%	%	ppm	ppm	ppm	ppm	ppm	%	ppm	ppm	cal/g
Clam, tongues	25.0	62.9	1.4	0.05	0.46	0.09	102	40	3	3	2.5	1.2	0.99	0.61	4961
Fish, Bonita steak	29.8	81.5	13.7	0.26	0.98	0.11	114	30	5	1	0.0	0.9	6.32	0.14	5972
Fish, Capelin, No heads / tails	20.4	74.3	18.5	1.35	1.60	0.14	262	60	3	10	0.0	0.9	1.35	0.19	5829
Fish, Capelin, whole (avg last 10 in 2014-2015)	19.0	75.6	16.5	1.76	1.91	0.16	92	71	3	4	0.1	1.0	1.52	0.15	5796
Fish, Glass Minnows	20.3	84.3	7.5	3.35	2.28	0.20	40	141	1	10	0.0	1.1	1.91	0.53	4777
Fish, Herring, whole (avg last 10 in 2014-2015)	29.9	54.8	37.0	1.51	1.45	0.13	69	60	3	4	0.1	0.7	2.15	0.12	6630
Fish, Lake Smelt, whole, avg	17.6	75.2	17.1	1.93	1.66	0.12	30	100	2	8	0.1	0.9	1.71	0.08	5727
Fish, Mackerel, whole, avg	27.3	79.2	11.0	1.43	1.68	0.16	114	47	5	2	0.2	1.0	4.17	0.21	5562
Fish, Sardine, whole	45.7	36.6	61.0	0.80	0.87	0.07	62	43	2	3	0.2	0.5	1.79	0.57	7418
Fish, Silversides, whole	26.1	59.1	27.1	2.04	1.75	0.18	34	93	2	5	0.3	1.0	1.73	0.10	6046
Fish, Trout, Idaho Rainbow	26.2	61.3	30.5	2.10	1.83	0.10	105	138	4	3	0.2	0.8	1.20	0.08	6180
Fish, Tuna, trimmed	25.5	98.8	1.2	0.03	1.07	0.12	49	14	2	0	0.1	0.9	6.11	0.09	5391
Gel, Mazuri 5AB0 Aquatic	22.6	64.0	16.7	2.15	1.54	0.14	367	312	19	110	0.3	0.7	1.21	4.99	5364
Krill, Pacifica	16.9	79.7	6.5	1.98	1.51	0.46	36	93	66	4	0.2	1.7	4.97	0.21	5374
Krill, Superba	21.0	61.7	19.8	1.41	1.52	0.36	30	44	84	2	0.2	1.7	1.54	0.17	5978
Prawn, no head, tail, or skin	22.2	94.7	3.5	0.35	1.36	0.21	91	68	25	3	0.1	1.3	2.08	0.14	5495
Prawn, whole	23.6	89.0	5.1	1.08	1.32	0.22	147	68	40	5	0.0	1.1	2.13	0.38	5144
Shrimp, Mysid, whole, avg	18.1	68.2	24.7	1.52	1.20	0.14	48	73	30	7	0.3	0.9	3.93	0.19	5956
Shrimp, White, no heads/ tails	22.3	85.4	3.5	0.74	1.17	0.16	105	49	23	14	0.0	0.8	1.21	0.15	5234
Shrimp, White, whole	19.7	88.4	4.1	0.37	0.80	0.14	65	52	11	7	0.2	1.1	1.35	0.19	5436
Squid, De-penned	18.9	85.1	4.5	0.08	1.35	0.21	9	71	105	2	0.0	1.5	2.98	0.06	5400
Squid, Humboldt	25.4	73.7	21.2	0.06	1.19	0.20	32	71	56	2	0.2	1.5	5.02	0.27	6156
Squid, whole (avg last 10 in 2014-2015)	18.9	80.5	6.7	0.13	1.12	0.25	16	81	183	5	0.1	1.9	3.47	0.22	5458

DESIGNING AN ONLINE HAND-REARING RESOURCE CENTER: FIRST STEPS

Gail E. Hedberg, RVT[1]*, Kimberly Thompson, DVM[2], Laurie J. Gage, DVM[3], Louise Peat[4], Kimberly Robertson[5], and Ellen S. Dierenfeld, PhD[6]

[1]Safari West, Santa Rosa, CA
[2]Michigan State University, East Lansing, MI
[3]USDA APHIS Animal Care, Center for Animal Welfare, Kansas City, MO
[4]Avian Rearing Resource, Burford, Oxfordshire, UK
[5]Ellen S. Dierenfeld, LLC, St. Louis, MO

In 2014, Safari West and the Safari West Wildlife Foundation (Santa Rosa, CA) hosted "The Inaugural AZVT (Association of Zoo Veterinary Technians) Focus Group: The Art and Science of Hand-Rearing", a symposium that was attended by 80 zoo professionals from around the globe. With the initial focus on carnivores and hoofstock, twenty international speakers provided their insights into both historical as well as new techniques. Discussions of hand-rearing zoo animals ranging from milk and colostrum composition to the special physiology and behaviors of neonates, and techniques for monitoring success. As a direct follow-up from the meeting, a steering committee comprising members from the zoo, wildlife rehabilitation, veterinary, and academic communities outlined criteria and developed a working model for data collection into a web based hand-rearing resource center. Phase I online data collection focuses on select mammalian species, working initially through SSPs, TAGs, AAZV, and AZVT communication liaisons for dissemination, followed by review and summarization of details of formulae and supplements, weight gains, developmental milestones, medical history and support materials. Additionally, we are partnering with the Avian Resource Center (http://www.avianrearingresource.co.uk/index.php; Burford, Oxfordshire, UK) in making available avian incubation and hand-rearing summaries that have already been compiled. Working collaboratively with aviculturists, we envision expanding these baseline incubation and rearing protocols and formats, as well as records of developmental milestones, for broader application to reptiles and amphibians.

EFFECT OF DIETARY SOLUBLE FIBER ON GUT MICROBIOTA IN THE SUGAR GLIDER (*PETAURUS BREVICEPS*): A PILOT STUDY

Lindsey Solden[1], Ellen S. Dierenfeld[2]*, and Kelly Wrighton[1]

[1]Department of Microbiology, The Ohio State University, Columbus, OH, USA
[2]Ellen S. Dierenfeld, LLC, St. Louis, MO, USA

Introduction

The sugar glider (*Petaurus breviceps*) is an exudativore in nature, eating plant gums, saps, resins, manna, and nectars as well as insect-based honeydew and lerp, with proportions of various ingredients highly dependent on seasonality and locale (Smith, 1982; Howard, 1989). Despite a well-developed cecum that could, in theory, harbor microbial populations with fermentative capabilities (Hume, 1999), a majority of captive gliders are fed diets comprising a high proportion of simple sugars such as nectars and domestic fruits (Dierenfeld et al., 2006). This investigation was undertaken to examine effects of added soluble dietary fiber on gut microbial populations in sugar gliders.

Materials and Methods

Animals

Twelve adult, pair-housed non-breeding gliders were utilized (n=6 study units); four pairs were male-female, with one female and one male pair. Animals (average weight 69.0 ± 9.7 g) were maintained in adjacent coated-wire cages measuring 46 X 46 X 75 cm (WLH), with a plastic catch tray underneath. Enclosures were furnished with flannel-lined hanging pouches, plastic hanging toys, an exercise wheel, and drip bottle drinkers. No special lighting regimen was used; the room was exposed to a normal daylighting cycle (April/May, northern hemisphere) through large windows, and temperature ranged from 18 to 22°C. Locations of cages were rotated weekly to minimize possible effects of light on appetite/activity. Gliders were weighed weekly (as pairs) on a platform scale (Model 3828, Taylor Precision Products, Las Cruces, NM USA) to 0.1 g, and all remained healthy throughout the trial.

Diets

Each glider pair was fed three diets (control, low fiber or high fiber) for a period of one week, with a one-week acclimation period between. Control diets consisted of extruded dry pellets (Glide-R-Chow™, from Pocket Pets™ @www.SugarBears.com) and a powdered supplement (Critter Love® HPW Complete, St. John's, FL); previous intake and digestion trials established dry matter intake (DMI) per pair as ~7 g. Conservative low and high soluble fiber diet treatments (~550 or 800 mg acacia gum + pectin – targeting 7.5 and 10% of DMI , respectively, per glider pair) - were developed by adding known quantities of dry acacia gum and apple pectin to the powdered supplement. Powders were blended with water as per label instructions, a food coloring dye was added to identify different treatments, and the liquid supplements were frozen in ice cube trays to provide consistent meal sizes. The acacia gum product used (Swanson Health Products, Fargo, ND) contained 500 mg per capsule, along with microcrystalline cellulose, magnesium stearate and/or silica and stearic acid, and the apple pectin supplement (Swanson Health Products, Fargo, ND) contained 300 mg pectin per capsule, with a rice flour carrier.

Fecal Collection and Analysis

Fecal samples were collected aseptically on the sixth and seventh days of each diet treatment by lining the cage bottom with autoclaved aluminum foil one hour prior to feeding in the evenings, and collecting fresh droppings, using sterile forceps, into cryotubes within 1-2 hours after feeding commenced. Samples were frozen until overnight shipment to the Ohio State University. DNA was extracted from day seven samples (~0.15 g feces) of each treatment (in duplicate as possible) using a MoBio PowerSoil DNA Isolation Kit following the kit protocol, yielding an average of 60 ng/µl. The V4 region of the 16S rRNA gene was PCR amplified (30 cycles) and sequenced with Illumina MiSeq by Argonne National Laboratory, creating a unique barcode to identify bacteria and archaea present in each fecal sample.

Statistics

Community data for each sample were analyzed using the Bray-Curtis similarity metric and plotted using R (version 3.1.3) or the program Primer (Clarke, 1993). As a result of the conspicuously low abundance of the highly dominant organism of all other samples, cage 5 was removed from further analysis. The average abundance of each OTU per treatment was used to represent the number of OTUs, and all OTUs with an average >0.001% relative abundance were included. Visual interpretations were validated statistically using ANOSIM in Primer (Clarke, 1993) at $P < 0.05$.

Results and Discussion

OTU is an operational definition of a bacterial or archaeal "species" used by microbiologists. Microbiologists refrain from using the term species unless validated using other metrics. Throughout this study, we use an OTU level (>97%) designation to discern different microbes from one another.

Diet and Cage Effects

Using non-metric multidimensional scaling (NMDS), with each colored dot representing the community from a single sample, the distance between each point visually represents the level of similarity between the communities. The high stress of the two NMDS plots (Figures 1 and 2) indicates that the similarity of the samples is not completely represented in two dimensions, and interpretations based solely on this clustering should be avoided. We found that dietary fiber content did not structure the microbial community in feces during the time measured (Figure 1), verified by lack of clustering by fiber treatment (similar symbols did not cluster together) and statistically. Similarly, cages did not impact the structure of the fecal microbiome (Figure 2). The distances within a group of clusters (e.g. filled circles, cage 2) were not visually different from other cages (e.g. filled squares, cage 1); this interpretation was also validated statistically.

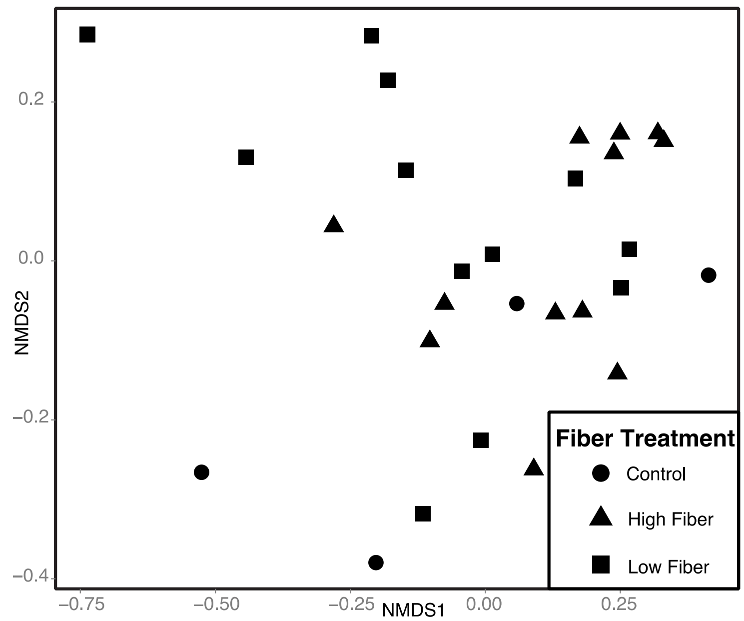

Figure 1. NMDS plot using Bray-Curtis distance for the 16S rRNA gene relative abundance data. Stress: 0.14. The fecal microbial communities (each sample represented by a point) do not cluster by fiber treatment.

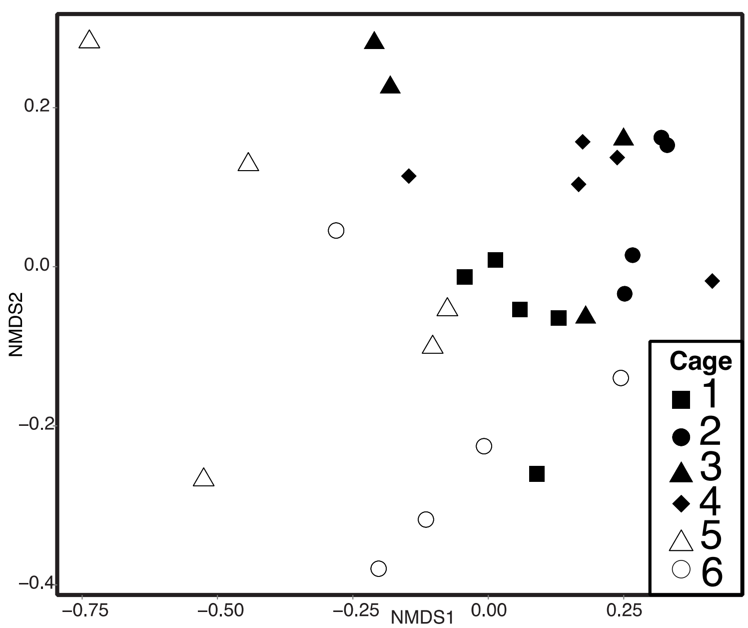

Figure 2. NMDS plot using Bray-Curtis distance for the 16S rRNA gene data set. The fecal microbial communities do not cluster by cage consistently, evidenced by the fact that the samples (points) are not clustered together by symbol.

OTU Overlap

Certain OTUs are shared across treatments (Figure 3). There were on average 153 core microbes shared over all treatments. Fiber treatments shared 70 OTUs; the High Fiber treatment had 22 unique satellite OTUs whereas the Low Fiber treatment had 34 unique satellite OTUs. Notably, only 1 OTU was identified unique to the baseline treatment that wasn't below detection in the high or low fiber diets.

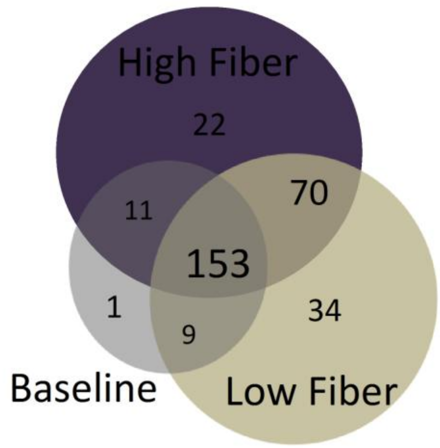

Figure 3. Diagram depicts numbers of OTUs detected in fecal samples from sugar gliders fed a baseline diet, compared with treatments including 7.5 % (Low Fiber) or 10% (High Fiber) added soluble fiber. Numbers are associated with diversity (total), overlap (shared) and unique microbial species identified in the samples.

OTU Change with Fiber Addition

While the entire microbial community failed to show a response to diet, some members of the community did change in abundance with diet (Figure 4). For example, an uncultivated *Bacteroides* sp. increased the most with the high fiber treatment (8.8%; bottom purple bar), followed by a *Succinivibrio* sp. (+2.7% of the high fiber treatment), and a *Faecalibacterium* sp. (+1.5% with high fiber).Typically in microbiome nutritional studies, treatment effects of at least a log-fold change are reported; none of the changes in this study were log-fold, and we cannot say whether or not this is a positive or negative change in the microbiome.

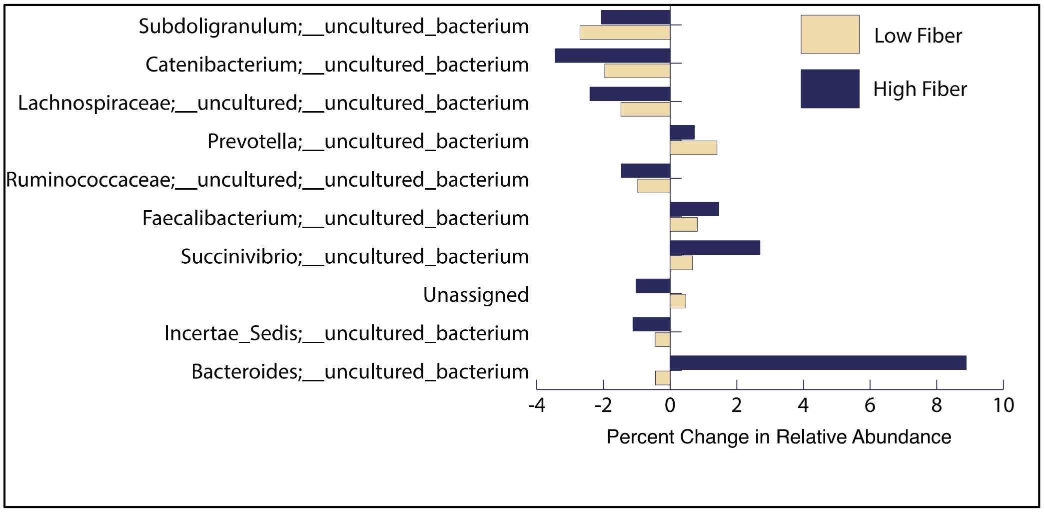

Figure 4. Bar chart representing the change in relative abundance of organisms that most responded to the addition of fiber. An average abundance of each OTU was calculated for each treatment and then compared to baseline.

With recent advancements from the Human Microbiome Project, we have learned that diet alters our gut microbiome and plays a role in our gastrointestinal health (David, 2013, Clemente, 2012). However the role of diet in gut health of many captive animals in zoos and private settings remains uncharacterized, as do normal microflora and optimal levels and types of dietary fiber. Here we explore the effect that increased fiber in the diet may have on the fecal microbial communities of sugar gliders. The inclusion of highly fermentable gel-forming dietary fibers at concentrations ranging from ~5 to 20% of dry matter has been associated with health benefits in a variety of animal and human models, impacting lipid metabolism, glucose tolerance, and absorption of minerals such as Ca and Mg (Levart *et al.*, 1991; Howard *et al.*, 1995). Altered gut microbial populations linked with fermentable dietary fiber are associated with suppression of potentially pathogenic bacteria as well as enhanced cecal mucosal tissue development (Howard *et al.*, 1995).

Captive sugar gliders may also benefit from diets containing higher soluble fiber than currently fed. Addition of up to 10% dietary fiber (as pectin plus gum Arabic) did not alter intakes or fecal consistency. Although microbial changes due to diet treatment were not of a statistically significant magnitude in this pilot study, genomes of closely related microbial genera to those taxa displaying change (Figure 4) contain the potential for fibrolytic activity (including beta-glucosidase, pectinesterase, endoglucanase, and beta-xylosidase). Assuming these enzymes may also be associated with microbial taxa identified in these animals, we speculate that sugar gliders may indeed have the microbial and anatomical capacity for increased fermentation of soluble dietary fibers. Longer trial periods, and/or increased levels in fiber treatments, could result in greater impact on the microbiome which, in turn, may improve digestive physiology and overall health in this species.

Conclusions

1) The most dominant Bacteria and Archaea were shared across the three treatments, with slight changes in abundance. Notably these are also dominant genera observed in fecal samples from other mammals, including:

- a *Bacteroides* sp. (uncultured bacterium from the Bacteroidaceae family)
- a *Prevotella* sp. (uncultured bacterium from the Prevotellaceae family) and
- a *Subdoligranulum* sp. (uncultured bacterium from the Ruminococcaceae family)

2) In this study, we could not statistically discern differences in fecal microbiomes by diet or by cage, although it was clear that one set of samples contained outliers, thus were not included in the analysis. Exclusion was largely attributed to a significant decrease in the most dominant organism found in all other samples.

3) All treatments shared 153 core microbial members, but certain microorganisms were only detected on specific diets.

4) Small changes in abundance of some specific microorganisms in response to diet treatment compared to baseline were observed; due to the limited magnitude of change, we cannot say whether the microbiome is negatively or positively affected. It is possible that longer treatments or increased fiber levels or types could result in greater impact on the microbiomes of the sugar glider.

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EVALUATING THE NUTRITION OF A FLOCK OF NON-BREEDING GREATER FLAMINGOS (*PHOENICOPTERUS RUBER ROSEUS*) AT THE SAN DIEGO ZOO SAFARI PARK

Michael L. Schlegel, PhD, PAS, Dipl. ACAS-Nutrition[1]*, Angela Ray, BS[2], and Jennifer Tibbot, BS[2]

[1]Nutritional Services Department, San Diego Zoo Global, San Diego, CA, USA.
[2]Bird Department, San Diego Zoo Safari Park, Escondido, CA, USA.

Abstract

A flock of 150 greater flamingos (*Phoenicopterus ruber roseus*) at the San Diego Zoo Safari Park were moved to a new enclosure in 2010 to provide them a larger exhibit and to promote the breeding of lesser flamingos (*Phoeniconaias minor*) that shared the previous exhibit. Since the greater flamingos moved, they have not laid eggs through June 2015. A nutrition review of the greater flamingos was conducted in 2014 and 2015 and included an evaluation of the nutrient composition of the diet, diet disappearance studies, body weight monitoring, and review of blood minerals and vitamins. The greater flamingos received a fine-grounded custom-manufactured diet that has been fed for more than 15 yr without changes in nutrient specifications. The diet was lower in vitamin A and E than desired and higher in Se than needed. The two diet disappearance studies suggested that an adequate amount of food was disappearing to meet the birds' energy needs. The greater flamingos had lost an average 13.8% of their body weight between 2010 and 2014 and continued to lose weight in 2015. The serum minerals and vitamins were within reference values except, whole blood selenium was greater. This nutrition review suggested that the current dietary concentrations of vitamin A, vitamin E, and selenium should be evaluated.

Introduction

In January and February, 2010, the 150 bird flock of greater flamingos (*Phoenicopterus ruber roseus*) at the San Diego Zoo Safari Park were moved from an enclosure (~3531 m^2, 0.35 ha, 0.87 ac) they shared with ~ 100 lesser flamingos (*Phoeniconaias minor*) to a new and larger (~18,635 m^2, 1.86 ha, 4.6 ac) exhibit. The goals of moving the greater flamingos were to provide them a larger exhibit and to promote the breeding of the lesser flamingos. The lesser flamingos did start to breed in 2010 and have produced chicks each year through 2014.

From 1998 to 2009 the greater flamingo flock produced 173 chicks. Since the birds moved, they have not produced any eggs or chicks from 2010 to June 2015. The plan for this nutrition review was to 1) evaluate the nutrient content of the diet fed, 2) conduct a diet disappearance study, 3) monitor body weights, and 4) collect blood samples to evaluate nutrients.

Materials and Methods

Diet Review

The greater flamingo flock received a diet of 91.5% San Diego Zoo Global (SDZG) Flamingo 24 Fine (Western Milling, Goshen, CA 93227) and 8.5% Mazuri Waterfowl Maintenance/Breeder (Purina Mills, LLC., St. Louis, MO 63116). The waterfowl floating diet was provided to attract the birds and to get them close enough for daily visual evaluations. Samples of SDZG Flamingo

24 fine diets were collected (May and December, 2014) for nutrient analysis at commercial laboratories (Dairy One, Ithaca, NY 14850; Michigan State University DCPAH, East Lansing, MI, 48910). The results were compared to the product specifications and the Flamingo Husbandry Manual recommendations (Brown and King, 2005).

Diet Disappearance Study

Because there had been some weight loss recorded in a few birds that had been handled for medical or other reasons, two diet disappearance studies was conducted. During the first week (Study 1, 7 d), Animal Care staff fed the SDZG Flamingo 24 Fine diet in the building attached to the exhibit as they usually do and tossed the waterfowl diet on the water near shore. To determine if the birds did not like going into the building to eat, in Study 2, the flamingos were fed in 10 grey plastic tubs on the shoreline for 7 d and again the waterfowl diet was tossed on the water near shore. The diet offered was weighed daily and Animal Care staff collected and weighed the remaining diet, orts, the next day prior to feeding. The amount of food that disappeared was compared to a suggested DM intake of 2-4% of body weight (Brown and King, 2005) and to maintenance energy requirements for zoo (ME kcal/d = 115 x $BW,kg^{0.729}$; Robbins, 1993) and free-ranging (ME, kcal/d = 2.51 x BW, $g^{0.681}$, all birds equation; Nagy et al., 1999) birds.

Body Weight Monitoring

With the heightened concern that the greater flamingo flock had not been reproductive for more than 4 yr, three pairs of birds were removed from the new exhibit and returned to their old exhibit with the lesser flamingos in March 2014. In the process of moving the birds, it was realized that 2.2 birds had an average weight loss of 25% compared to January/February 2010. Opportunistic weights and weights during medical procedures were collected in December 2014 and May 2015 and compared to 2010 when the birds were moved to the new exhibit. Additionally, 15 (6.9) greater flamingos were introduced into the flock in 2011 to try to stimulate breeding. For 6 birds that were weighed 2015 plus 2 resident birds, weights collected in 2011 were used for calculate changes in body weight.

Serum Nutrient Evaluation

To evaluate the nutritional health of the birds, an initial three (0.3) greater flamingos had blood collected when they were moved back to their old exhibit in March 2014. Additional samples were collected from three (2.1) greater flamingos (vitamins only) in November 2014 and 13 (4.9) greater flamingos in December 2014. Blood was collected into acid-washed tubes (Becton, Dickinson, and Co., Franklin Lakes, NJ, 07417 USA) for serum mineral (Ca, Cu, Fe, Mg, P, K, Na, Se, Zn) and vitamin analysis (A and E) and K2 EDTA tubes (Becton, Dickinson, and Co., Franklin Lakes, NJ, 07417 USA) for whole blood selenium analysis. Banked serum samples from 30 flamingos collected in 2010 were analyzed for vitamin A (13 samples) and vitamin E (26 samples) to compare with samples collected in 2014.

Blood samples were analyzed at a commercial laboratory (CAHFS, Davis, CA 95617). Blood nutrient concentrations were compared to previous results of SDZG flamingos (unpublished data) and to those in the literature for flamingos (Benato et al., 2013; Brown and King, 2005; Dierenfeld, 1989; Schlegel et al., 2005; Teare, 2013a,b) and poultry (Puls, 1988). A paired t-test

(MS Excel) was used to determine if serum vitamin concentrations had changed over the 4 yr since the birds were in the new exhibit (2010 versus 2014) using the banked serum samples.

Results and Discussion

Diet Review

The custom SDZG Flamingo 24 diet is being fed to the greater flamingos and three other species of flamingos (Caribbean, *Phoenicopterus ruber ruber*; Chilean, *Phoenicopterus chilensis*; and Lesser) at the San Diego Zoo and San Diego Zoo Safari Park. The greater and lesser flamingos receive the fine particle-size product and the Caribbean and Chilean flamingos receive the productin a pelleted form. All but the greater flamingos have been reproductive in the last 4 yr. The SDZG Flamingo 24 diet specifications meet, exceed, or are within the range of nutrient concentrations recommended by the husbandry manual (Brown and King, 2005) except vitamin A (Table 1). The vitamin A specification (11,111 IU/kg DM) is 45% less than suggested by the husbandry manual (20,000 IU/kg DM) and has been formulated this way for more than 10 yr. Vitamin A deficiencies have not been a significant finding on flamingo necropsies. The lots of SDZG Flamingo 24 diet tested (Table 1) were slightly below product specifications for protein, calcium and phosphorus, but were within ranges suggested by Brown and King (2005). The sample analyzed for vitamin E (162.2 IU/kg) was more than 50% below the product specification (333 IU/kg) and below the suggested concentration by Brown and King (2005). The selenium concentration of the one sample analyzed (1.01 mg/kg DM) was 3 times the product specification (0.33 mg Se /kg DM), but toxicity starts at 5 mg Se/kg DM (NRC, 1994). Analysis of B vitamins in 2013 met or exceeded the product specifications, but vitamin A concentrations were lower than product specifications (data not shown). Based on husbandry manual guidelines, there are areas in the product specifications that need to be adjusted.

Diet Disappearance Study

Ninety-seven percent and 72% of the flamingo diet that was fed disappeared when offered in the barn or on the shore, respectively (Table 2). The birds readily consumed the waterfowl diet when tossed to them on the water. When the birds were fed in the building, 22% more feed disappeared than when fed on the shore. Based on the diet disappearing, the amount of dry matter consumed per bird was 7.5% and 6.1% of body weight for a 3-kg flamingo. This consumption is higher than the suggested 2-4% in the husbandry manual (Brown and King, 2005). Estimated energy consumption of both diet disappearance studies suggests that more than adequate amounts of diet were consumed to support the maintenance energy requirement of a 3-kg bird at a zoo or free-ranging (Table 2). It should be noted that these studies could not account for consumption by native wildlife (mallards, *Anas platyrhynchos*; and coots, *Fulica americana*) or the feed that got pushed out of the feeders and dropped in the water and not consumed.

Body Weight Monitoring

Three of the greater flamingos that were returned to their old exhibit in March 2014 were weighed again in November 2014 and had averaged a 21.1% body weight gain. In December 2014, when the main flock was being manually restrained for blood collection, 4.9 birds were weighed and the average weight loss was 13.8% (range 26.8% loss to 1.7% gain) compared to 2010. A female greater flamingo that arrived in 2011 to stimulate the flock to breed lost 18.5% from its arrival weight to 2014. To try to promote weight gain, the waterfowl breeder that was used previously, was replaced with Mazuri Flamingo Breeder (Purina Mills, LLC., St. Louis, MO 63116) and 25% of the SDZG Flamingo 24 fine was replaced with the flamingo breeder increasing the crude protein 9%, the fat 13.6%, with a 1.8% increase in total energy. In May 2015, 40 greater flamingos were weighed again to see the effect of the diet change. Of the 30 (11.19) birds that moved in 2010 and had weights recorded in 2015, the average weight loss was 14.4% (range 28.1% loss to 7.4% gain). Of the 6 (3.3) birds that joined the flock in 2011 plus two resident (0.2) birds, the average weight loss was 6% (range: 18.5% loss to 6.5% gain) from 2011 to 2015. Two (0.2) birds that were weighed in May 2015 had not been weighed in 2010 or 2011 to allow similar comparisons.

Serum Nutrient Evaluation

The initial three (0.3) greater flamingos, sampled in April 2014, had greater serum P and K, compared to greater flamingo reference ranges (Teare, 2013a; Table 3). Selenium concentrations were higher in whole blood from both groups of Safari Park greater flamingos than the greater flamingos in the study by Benato et al. (2013) from the United Arab Emirates (UAE) and domestic poultry (Puls, 1988). The higher Se concentrations can be explained by the higher dietary Se. Serum Ca, Cu, Mg, Na, and Zn from the Safari Park flamingos were similar to reference values (Puls, 1988; Teare, 2013a,b).

The vitamin A concentrations in the serum from the three (0.3) flamingos bled in April 2014 were lower than those collected in November and December and lower than serum collected in 2010, but as a group, the vitamin A concentrations from 2014 were similar to 2010 and the reference values (Table 4). The serum vitamin E concentrations in all of the samples collected in 2014 were similar to 2010 and the reference values. Thirteen (3.10) flamingos had serum vitamin A and E samples from 2010 and 2014 to allow direct comparisons. There were no differences ($P = 0.23$) in vitamin A concentrations between 2010 and 2014 (0.59 mg/L versus 0.63 mg/L, respectively). The vitamin E concentration in the serum from 2014 (30.8 mg/L) was 15% greater ($P = 0.02$) than the serum from 2010 (26.5 mg/L).

Next Steps

It has been difficult to explain why, given the apparently adequate diet disappearance and appropriate serum nutrient profiles, the birds continued to lose weight. One explanation for the weight loss is due to increased activity. The greater flamingos were moved to an exhibit that was more than five times larger and the flamingos are spending more time swimming thus increasing their energy expenditure. The greater flamingos that moved back to their old exhibit regained most of the lost weight. The current SDZG Flamingo 24 diet appears to meet the nutritional needs of the Caribbean, Chilean and lesser flamingos as evidence by reproductive successes. Historically, the greater flamingos had reproductive success on the SDZG Flamingo 24 diet, but this nutrition review suggests that the current concentrations of vitamin A, vitamin E, and selenium should be evaluated.

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Table 1. Nutrient recommendations, product specifications and recent laboratory analysis of San Diego Zoo Global Flamingo 24 fine.

Nutrient	Husbandry Manual Recommendations[1]	SDZG Flamingo 24 Fine Specifications	Sample 2014.080	Sample 2014.212
Crude Protein, %	20-40	> 26.67	25.5	25.9
Lysine, %	NR[2]	1.3	-[3]	-
Methionine, %	NR	0.6	-	-
Methionine + Cystine, %	NR	1.0	-	-
Acid Detergent Fiber, %	NR	< 2.2	5.4	9.7
Crude Fat, %	NR	> 4.4	4.7	4.2
Linoleic acid, %	> 1	> 1.22	-	-
Ash, %	NR	< 11.1	10.7	9.5
Energy, ME, kcal/g	3.00	2.96[4]	-	-
Calcium, %	1-3	2.33-2.77	2.09	1.87
Phosphorus, %	0.5-1	> 0.94	0.79	0.73
Chloride ion, %	0.2	NS[5]	0.63	0.26
Magnesium, %	0.1	> 0.28	0.28	0.25
Potassium, %	0.2	> 1.11	1.29	1.11
Sodium, %	0.2	> 0.28	0.35	0.22
Cobalt, mg/kg	0.2	NS	0.71	-
Copper, mg/kg	15	> 16.7	23	29
Iodine, mg/kg	0.5-1.0	> 1.11	-	2.27
Iron, mg/kg	50-100	> 111	320	268
Manganese, mg/kg	50-75	> 77.8	156	132
Selenium, mg/kg	0.2-0.3	> 0.33	-	1.01
Zinc, mg/kg	50-100	> 111	173	165
Vitamin A, IU/kg	20,000	> 11,111	-	-
Vitamin D, IU/kg	2000	> 2440	-	-
Vitamin E, IU/kg	200	> 333	-	162.2
Vitamin K, mg/kg	1	> 2.22	-	-
Thiamin, mg/kg	5-10	>5.6	-	-
Riboflavin, mg/kg	10-20	>8.9	-	-
Niacin, mg/kg	25-35	>111	-	-
Pyridoxine, mg/kg	10-20	>6.7	-	-
Vitamin B12, mg/kg	4	>33.4	-	-
Pantothenic Acid, mg/kg	10-20	>22.2	-	-
Folic acid, ppm	NR	NS	-	-
Biotin, ppb	NR	>278	-	-
Choline, ppm	NR	>1667	-	-

[1]Brown and King (2005), [2]No recommendations given, [3]Not analyzed, [4]Calculated using domestic poultry (NRC, 1994), [5]No specification given.

Table 2. Food disappearance and intake of dry matter and energy from a flock of greater flamingos (*Phoenicopterus ruber roseus*) when fed in the barn or on the exhibit shoreline at the San Diego Zoo Safari Park.

Item	**Study 1: Building**	**Study 2: Shore**
SDZG Flamingo 24 fine		
Offered, kg	36.44	39.35
Orts, kg	1.00	10.85
Consumed, kg	35.44	28.50
Mazuri Waterfowl Breeder		
Offered, kg	3.54	3.62
Orts, kg	0	0
Consumed, kg	3.54	3.62
Intake per bird (157 birds in the flock)		
As-fed intake, kg	0.248	0.204
DM intake, kg[1]	0.223	0.184
DM intake, % of body weight[2]	7.45	6.14
Energy intake, kcal ME[3]	672	566
Requirement, Zoo, kcal ME/d[2,4]	253	
Requirement, Free-ranging, kcal ME/d[2,5]	585	

[1]Assumes 90% DM.

[2]Based on a 3.0 kg flamingo.

[3]SDZG Flamingo 24 Fine, 2.66 kcal ME/g as-fed; Mazuri Waterfowl Breeder, 3.22 kcal ME/g as-fed.

[4]ME kcal/d = 115 x BW,kg$^{0.729}$ (Robbins, 1993).

[5]ME, kcal/d = 2.51 x BW, g$^{0.681}$ (all birds, Nagy et al., 1999).

Table 3. Blood mineral concentrations of San Diego Zoo Safari Park greater flamingos (*Phoenicopterus ruber roseus*) sampled in 2014 compared to concentrations found in the literature.

	SP 0.3 Greater Flamingos[1] 3-4 April 2014		**SP 4.9 Greater Flamingos[1]** 9 December 2014		**UAE 21.15 Greater flamingos[2]**		
Element	**mean**	**range**	**mean**	**range**	**mean**	**range**	**Reference values**
Ca, ppm[3]	98.7	91-110	116	98-130	-[5]	-	102-180[6]
Cu, ppm[3]	0.37	0-0.67	0.33	0.26-0.48	0.36	0.21-0.52	0.20-0.45[7]
Fe, ppm[3]	1.07	0.6-1.8	1.02	0.65-2.30	-	-	NR[8]
Mg, ppm[3]	22.3	20-24	26.2	22-29	-	-	20.5-43.5[9]
P, ppm[3]	77.7	65-91	47.5	22-78	-	-	21-60.9[6]
K, meq/L[3]	5.3	2.6-6.9	3.28	2.2-4.0	-	-	1.8-3.2[6]
Na, meq/L[3]	143.3	140-150	160.8	150-180	-	-	145-161[6]
Se, ppm[4]	0.65	0.62-0.69	0.78	0.67-1.30	0.21	0.12-0.32	0.13-0.20[7]
Zn, ppm[3]	1.80	1.1-2.2	2.40	2.0-3.3	2.08	1.39-2.71	1.85-3.40[7]

[1]San Diego Zoo Safari Park.
[2]United Arab Emirates, Benato et al., 2013; conversions used: copper µM x 0.063546 = mg/L; selenium µM x 0.07896 = mg/L; zinc µM x 0.06538 = mg/L.
[3]Serum.
[4]Whole blood.
[5]Not analyzed.
[6]Teare, 2013a.
[7]Puls, 1988.
[8]No reference value available.
[9g]Teare, 2013b.

Table 4. Serum vitamin A and E concentrations of greater flamingos (*Phoenicopterus ruber roseus*) at the San Diego Zoo Safari Park sampled in 2010 and 2014 compared to concentrations previously analyzed at San Diego Zoo Global and those in the literature.

	Vitamin A, mg/L			Vitamin E, mg/L		
Species	***n***	**mean**	**range**	***n***	**mean**	**range**
SP Greater Flamingos (3-4Apr14)[1]	0.3	0.43	0.28-0.54	0.3	31.3	30-33
SP Greater Flamingos (29Nov14)[1]	2.1	0.78	0.68-0.90	2.1	22.0	21-24
SP Greater Flamingos (9Dec14)[1]	4.9	0.63	0.45-0.77	4.9	31.3	21-41
SP Greater Flamingos (2010)[1]	3.10	0.59	0.43-0.81	9.14	24.6	12-40
Referene values						
SP Greater Flamingos (1999-2002)[1]	4	0.58	0.51-0.62	4	24.2	18.4-28.7
DAK Greater Flamingos (1999-2003)[2]	-[6]	-	-	31	12.2	5.3-27.3
DAK Greater Flamingos (2004-2005)[2]	-	-	-	26	46.3	13.2-110.8
UAE Greater Flamingo, males[3]	21	0.46	0.26-0.77	21	12.8	4.6-19.1
UAE Greater Flamingo, females[3]	15	0.47	0.32-0.72	15	14.6	9.4-26.7
Flamingo (2 *Phoenicopterus* spp.)[4]	-	-	-	-	-	10.7-34.0
SDZG Lesser Flamingos (1995-1997)	-	-	-	27	22.4	1.3-57.7
UAE Lesser Flamingo, both[3]	14	0.39	0.32-0.52	14	12.7	7.1-18.0
Lesser Flamingos[5]	6	0.73	0.45-0.92	6	13.2	10.0-15.4
SDZ Caribbean Flamingos (1996)	-	-	-	28	46.8	16.8-73.1
Semi-free ranging Caribbean Flamingos[5]	53	0.046	0.00312-1.443	53	19.0	4.3-38.9
Caribbean Flamingos[5]	15	0.44	0.12-0.77	15	15.4	0.8-25
SP Chilean Flamingos (2003-2004)	50	0.82	0.45-1.47	50	58.2	24.9-140
Chilean Flamingos[5]	55	1.23	0.16-2.29	55	21.2	0.5-34.3

[1]San Diego Zoo Safari Park
[2]Disney's Animal Kingdom, Schlegel et al., 2005.
[3]United Arab Emirates; Benato et al., 2013; conversions used: vitamin A µM x 0.28645 = mg/L; vitamin E µM x 0.43071 = mg/L.
[4]Dierenfeld, 1989.
[5]Brown and King, 2005.
[6]Not analyzed.

EVALUATION OF A COMMERCIAL VITAMIN AND MINERAL SUPPLEMENT IN MILK REPLACERS ON SERUM NUTRIENTS IN PRE-RUMINANTS

Michele Gaffney, MS* and Michael L. Schlegel, PhD, PAS, Dipl. ACAS

Nutritional Services Department, San Diego Zoo Global, San Diego, CA, USA.

Abstract

Since opening in May 1972, the San Diego Zoo Safari Park has hand-raised hundreds of ruminant neonates. In order to improve success rates and generate appropriate protocols with any hand-reared neonate species, it is important to document each animal's daily status and changes implemented to husbandry practices with each experience. Milk formula composition and consumption, solid-food intake, body weights, and fecal output and consistency are all closely monitored by animal care staff. This paper evaluated the effectiveness of a commercially available vitamin and mineral supplement in milk replacers on serum and whole blood nutrient concentrations in pre-ruminant animals hand-raised between 01-Sept-13 and 01-Dec-14. The inclusion of the vitamin and mineral supplement in eight milk-replacer formulas increased the average dietary concentrations of copper, iron, vitamin E, and selenium by 77.5, 798.0, 391.0, and 81.0%, respectively. However, 50% of the formulas were still below the recommended concentration for both dietary copper and iron as suggested in NRC (2001). At least 91.6, 88.5, and 97.5% of the neonate hoofstock hand-raised on the formulas were within or above the adequate range for serum copper, iron, and whole blood selenium concentrations. Ninety-seven percent of the serum vitamin E concentrations were within the ranges reported for plasma vitamin E concentrations in five related families of artiodactyla.

Introduction

Following the decision to hand-raise an animal, usually due to dam neglect or medical issues with the calf, each hoofstock neonate was relocated from the exhibit to the Animal Care Center (ACC) at the San Diego Zoo Safari Park. The neonate was first examined by the veterinary staff and received treatment for any medical problems. Detailed records were kept on each animal, noting body weight, formula composition and intake, feeding frequency, urine output, and stool frequency and quality. The calves remained at the ACC until weaned at 3 to 4 mo of age.

Historically, milk-replacer formulas have been formulated to mimic values reported for the species dam's milk from available data or analysis, or the animals' are offered a suitable milk replacer from an appropriate model species. All of the neonatal ruminant milk formulas are deficient in iron and have been supplemented. The majority of the formulas are whole goat's milk-based with varying ratios of commercial milk replacers added. Two iron supplements have been used at the San Diego Zoo Safari Park. Vi-Sorbin (vitamin-iron preparation with sorbitol; Pfizer Inc., Pfizer Animal Health, New York, NY, 10017), and Lixotinic (vitamin and mineral supplement for horses, dogs, and cats; Zoetis Inc., Kalamazoo, MI, 49007). Visorbin was discontinued and Lixotinic was the suggested replacement. Lixotinic was used until concerns arose with feeding any products containing ruminant tissue to a ruminant animal. Lixotinic contained ruminant-derived liver paste. The concern was over the potential of exposing our animals to products from cattle infected with Bovine Spongiform Encephalopathy (BSE).

In 2005, a new liquid iron supplement from Kirkman Labs was identified as appropriate to use for ruminants. Each vitamin and mineral supplement was added daily to the first bottle for each neonate until it was weaned. With the Vi-sorbin product, each animal received 0.66 mg Fe/kg BW (0.33 ml/kg BW), and with Lixotinc each animal received at 1.1 mg Fe/kg BW (0.44 ml/kg BW). The dose established for the Kirkman's liquid Iron was 1.05 mg Fe/kg BW (0.3 ml/kg BW).

In May 2014, several species of neonatal ruminants that were being raised at the ACC, were reported to have low serum copper levels (< 0.7 mg/L and < 0.8 mg/L for sheep, and bovine and caprine, respectively, Table 1). The neonatal ruminants (n = 9) that were copper deficient received a treatment of copper sulfate solution in their bottles, daily for one month. The nine animals represented 39% of hand-raised animals that had blood mineral and trace mineral panels between April-September 2013. These same animals had follow-up lab results performed, and all but one animal's serum copper level was adequate (> 0.76 mg/L).

Maternal deficiencies of phosphorus, manganese, cobalt, copper, zinc, and selenium can result in deficiencies in the fetus and newborn calf (NRC, 2001). The fetus has the ability to concentrate some of these minerals, particularly copper and selenium providing some protection against marginal deficiencies in the dam (NRC, 2001). A number of ruminant species housed in large field exhibits (25-65 acres or 10-26 hectares) at the San Diego Zoo's Safari Park have been documented with chronic low-copper levels. This issue is currently being addressed with feeding a custom formulation of a High-Copper Herbivore pellet (40-50 mg/L) as compared to the custom standard High-fiber Herbivore pellet (20-30 mg/L). Affected animals can also be given a copper bolus opportunistically during a medical procedure requiring an immobilization. It is not evident if serum mineral or vitamin deficiencies seen with some of the neonatal ruminant species at the Safari Park are due specifically to a dam's mineral status, or formulas with dietary deficiencies, or if both issues will have to be addressed simultaneously to mitigate the problem.

Nutrition was asked to review the hand-rearing formulas (Table 2) for deficiencies and to consider dietary modifications to address the low serum copper. Vibra-Life Fortifier (Hampel Animal Care, Germantown, WI, 53022), a commercially available vitamin and mineral powder supplement was identified. A sample of this supplement was sent to an independent laboratory for analysis (Table 3).

Methods

The nutrient recommendations for dairy calves fed a milk replacer were deemed appropriate for the species of ruminants most commonly hand-raised at the ACC (Table 4). The supplement was added to the existing formulas used at the ACC at the recommended rate of 0.6 g per L of milk formula (including formulas containing a commercial milk replacer). This vitamin and mineral supplement was added daily to the first bottle for each neonate until the animal was weaned. Table 2 shows the nutrient concentrations of the supplemented formulas compared to the NRC (2001) recommended nutrient concentrations for dairy calves. Since the concentration of iron in the formulas was also increased with the new supplement, the Kirkland liquid Iron was discontinued to mitigate any excessive dietary iron. The normal protocol for hand-raising hoofstock was followed during the 15 month period when the use of the Vibra-Life supplement was implemented.

Blood samples were routinely taken after an animal had nursed formula for 24 h to determine if its immunoglobin G (IgG) levels were appropriate for determining passive immunity. Also, blood was drawn, by the veterinary staff, when animals were ill, and at four or more weeks of age when the animals were scheduled to receive a vaccine to protect against clostridium bacterium infection. Blood was collected into acid-washed tubes (Becton, Dickinson, and Co., Franklin Lakes, NJ, 07417, USA) for serum mineral, and vitamin E analysis and K2 EDTA tubes (Becton, Dickinson, and Co., Franklin Lakes, NJ, 07417, USA) for whole blood selenium analysis. The blood samples are analyzed for blood chemistry and serum-macro and trace-element screens, including calcium, phosphorus, iron, copper, magnesium, potassium, sodium, and zinc (California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, UC Davis, Davis CA, 95616). Analysis for vitamin E (serum) and selenium (whole blood) was often requested to help with assessing the health status of the neonates. Blood samples were collected when the animals were at least 4 wks old with most samples collected between 4 to 6 wks.

This post-natal period allowed for nutrient normalization in the tissue of the neonates receiving the supplement. Serum copper, iron, vitamin E and whole blood selenium were the nutrients are of particular interest because chronic deficiencies have been observed in Safari Park animals, and due to their importance for growth. The ranges of each of these four nutrients plus standard deviations were determined for 20 different species of ruminant hoofstock for the combined total of 39 individual animals (Table 4).

No statistical analysis to determine significant findings were performed. The objective was to evaluate if the supplement improved serum copper to adequate levels, and produced adequate serum iron and vitamin E, and blood selenium levels in pre-ruminant hoofstock.

Results and Discussion

Table 2 lists the nutrient concentrations of eight supplemented milk-replacer formulas used at the ACC, compared to the NRC (2001) nutrient recommendations for dairy calves. Although, all milk formulas are prepared to meet all the nutritional needs of the neonates, only minerals, trace minerals, selenium, and vitamin E are highlighted for the purpose of this project.

Table 1 lists the adequate serum or whole blood concentrations of copper, iron, vitamin E, and selenium in ruminants. Reference ranges from the California Animal Health and Food Safety Laboratory (Davis, CA) and Puls (1994) which are the reference values used by SDZG Veterinary and Nutritional Services Departments to evaluate the health status of animals.

Across all species (n = 20), the average for serum copper was 1.10 mg/L (range = 0.41-2.20 mg/L, S.D. = 0.34 mg/L), serum iron was 2.22 mg/L (range = 0.54-4.20 mg/L, S.D. = 1.04 mg/L), serum vitamin E was 2.97 mg/L (range = 0.36-6.80 mg/L, S.D. = 1.56 mg/L), and whole blood selenium was 0.38 mg/L (range = 0.15-0.67 mg/L, S.D. = 0.15 mg/L; Table 4).

Copper

The recommended concentration for copper in milk-replacer formulas for dairy calves is 10 mg/L (NRC, 2001). The copper concentrations in the eight formulas ranged between 3.97-12.83

mg/L, and were lower than the recommended concentration by 60%, to above the recommended concentration by 28% (Table 2). The formulas containing less than 10 mg/L were the Whole Cow's Milk:Powdered Goat's Milk 11:1, Whole Goat's Milk:Powdered Goat's Milk 18:1, Whole Goat's Milk:Doe's Match:Whey:Water 20:1:1:3, and Whole Cow's Milk (Table 2). When the Vibra-Life supplement was first used in September 2013, the first three formulas were not currently being fed, and subsequently were not evaluated for the copper concentration with an assumption the level would be sufficient. Adding the vitamin and mineral supplement to Whole Goat's Milk at the advised rate did not raise the copper concentration to the NRC (2001) recommendation; however, there were concerns increasing the rate of supplementation would increase the concentration of dietary vitamin A above the reported safe limit. The presumed safe limit for vitamin A is 66,000 IU/kg of dietary DM for lactating and non-lactating cattle (NRC, 2001), but safe limits specifically for young calves have not been established. Supplementation levels of several times the requirement are common in commercial milk replacers (NRC, 2001).

The animal with the lowest serum copper concentration (0.41 mg/L) was on the formula with a copper concentration of 8.80 mg/L. The highest serum copper concentration of 2.20 mg/L occurred in one animal that received the formula with dietary copper concentration of 12.83 mg/L. Puls (1988) list serum concentrations of 2.5 mg/L, 1.8 mg/L, and 5.0 mg/L as high, and 10.0 mg/L, 7.5 mg/L, and 20.0 mg/L as toxic for bovine, caprine, and ovine, respectively. Collectively, 91.6% or 100% of the 39 animals had adequate or greater serum copper concentrations when using the CAHFS lab (0.70 – 1.50 mg/L) or Puls (1988; 0.32 – 2.00 mg/L) reference ranges, respectively (Table 4).

Iron

The recommended concentration for iron in milk-replacer formulas for dairy calves is 100 ppm (NRC, 2001). The iron concentrations in the eight formulas ranged between 60-114 ppm, and are below the recommended concentration by 60%, to above the recommended concentration by 14.4% (Table 2). A dietary iron concentration of 95 ppm is recommended for growth in goats (NRC, 2007). A study by Lindt and Bloom (1994) suggested that a diet with 50 mg iron/kg DM was sufficient to support growth although the carcass remains pale (NRC, 2001). The formulas containing less than 100 mg/L were the Whole Cow's Milk:Powdered Goat's Milk 11:1, Whole Goat's Milk:Powdered Goat's Milk 18:1, Whole Goat's Milk:Doe's Match:Whey:Water 20:1:1:3, and Whole Cow's Milk (Table 2).

The animal with the lowest serum iron concentration (0.54 mg/L) was on the formula with a iron concentration of 107 mg/L, although other animals of the same species had adequate serum iron concentrations (1.3 mg/L or above, Table 4). The highest serum iron concentration of 4.2 mg/L occurred in one animal that received the formula with the highest dietary iron concentration of 114 ppm. Puls (1988) lists serum concentrations of 400-600 mg/L, and 1800-2500 mg/L as high and toxic, respectively, for bovine (Table 1). Collectively, 88.5% of the 39 animals were within or above adequate serum iron concentrations when referencing either CAHFS (1.30-2.50 mg/L) or Puls (1988; 1.30-2.50 mg/L) reference ranges, respectively (Table 1).

Vitamin E

The recommended concentration for vitamin E in milk-replacer formulas for dairy calves is 50 ppm (NRC, 2001). Vitamin E concentrations in the eight formulas ranged between 74.0-222.0

ppm, exceeding the recommended level by 150-400% (Table 2). Vitamin E is one of the least toxic vitamins due in part to its relatively low absorption (NRC, 2001). Toxicity studies have not been conducted with ruminants, but data from rats suggest an upper limit of approximately 75 IU/kg of body weight per day (NRC, 2001).

The average serum vitamin E concentration (2.97 mg/L) in the neonatal ruminants was similar to means seen in Dierenfeld (1989) for plasma vitamin E concentrations of 1.90, 1.92, 2.03, 2.09, and 3.01 mg/L, for camelidae, bovidae, giraffidae, cervidae, and tragulidae, respectively. The range in serum vitamin E concentrations seen in the neonatal ruminants (0.36-6.80 mg/L, Table 4) was also similar to the wide ranges seen in the Dierenfeld (1989) study, where plasma vitamin E ranges of 1.0-5.4, 0.5-8.5, 0.9-7.0, 0.4-6.7, 0.7-5.0 mg/L were reported for camelidae, bovidae, giraffidae, cervidae, and tragulidae, respectively.

The hand-raised animal with the lowest serum vitamin E concentration (0.36 mg/L) was on the formula containing the highest concentration of 222.0 mg/L; but there was another animal of the same species that had a serum vitamin E level of 2.70 mg/L. One animal's blood results came back with vitamin E as undetectable, and received the formula with a vitamin E concentration of 198 ppm; however, other animals of the same species had serum vitamin E concentrations between 1.6-5.4 mg/L.

Selenium

The recommended concentration for selenium in milk-replacer formulas for dairy calves is 0.3 ppm (NRC, 2001). The selenium concentrations in the eight formulas ranged between 0.67 – 1.10 ppm (Table 2), and are all above the recommended concentration by 223-367%. NRC (2001) states that chronic toxicity can occur when cattle are fed diets with 5 to 40 mg of selenium/kg (DM) for several weeks or months. Puls (1988) list serum concentrations of 0.8-2.5 mg/L and 3.5-4.1 mg/L as high and toxic for bovine, respectively, and 3.0 mg/L and above as toxic to capine. Collectively, 97.4% of 30 animal samples had more than adequate selenium concentrations when referenced with Puls (1988; 0.03 – 0.15 mg/L) for bovine, plus 80.0% and 20% had adequate, and more than adequate selenium concentrations for ovine (Table 4).

Summary

There will be many opportunities in the future allowing animal care staff to collect more information (i.e., milk formula composition, supplementation, and consumption, solid food intake, body weights, fecal output and consistency) and further improve hand-rearing successes with neonatal ruminants. A complicating factor affecting mineral, and vitamin status of a neonate animal is its health status. If an animal has a medical issue, its nutrient regulatory biochemical pathways may be compromised.

Although the majority of the animals had adequate serum copper and iron concentrations, based on the findings in this case study, dietary copper and iron need to be improved in four formulas, and an alternate commercial or custom vitamin and mineral supplement should be considered.

All eight formulas contained vitamin E and selenium concentrations in excess of the recommended concentrations for dairy calves (NRC, 2001). Serum vitamin E and whole blood selenium concentrations were adequate or above adequate for the majority of the hand-raised ruminant neonates.

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Table 1. Reference ranges of adequate serum copper, iron, and selenium concentrations for bovine, caprine, and ovine.

	Serum Copper		Serum Iron		Serum Selenium
Species	**CAHFS**[1]	**Puls**[2]	**CAHFS**	**Puls**	**Puls**
Bovine	0.80 – 1.50	0.32 – 1.20	1.30 – 2.50	1.30 – 2.50	0.025 – 0.150
Capine	0.80 – 1.20	0.80 – 1.20	1.50 – 2.50	nr[3]	nr
Ovine	0.70 – 1.50	0.70 – 2.00	1.70 – 2.20	1.66 – 2.22	0.08 – 0.50

[1]California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, UC Davis, Davis, Ca.; values reported as mg/L.
[2]Puls, 1988; values reported as mg/L.
[3]nr = Not reported.

Table 2. Nutrient composition (DMB) of selected milk-replacer formulas with VibraLife[1] vitamin and mineral supplement used for hand-rearing pre-ruminants at the San Diego Zoo Safari Park as compared to nutrient recommendations for dairy calves.

	Milk Replacer Formulas[2]								
Nutrient	**WGM**	**WGM:DM 11:1**	**WGM:DM 14:1**	**WGM:DM:H2O 15:2:1**	**WGM:DM 18:1**	**WGM:PGM 18:1**	**WCM:PGM 11:1**	**WGM:DM:WHEY:H20 20:1:1:3**	**Nutrient Requirements of Dairy Calf[5]**
Calcium, %	1.04	1.01	1.01	1.00	1.02	1.02	0.98	0.91	1.0
Phosphorus, %	0.86	0.84	0.84	0.84	0.84	0.85	0.80	0.72	0.7
Ca:P ratio	1.2:1	1.2:1	1.2:1	1.2:1	1.2:1	1.2:1	1.2:1	1.25:1	1.4:1
Magnesium, %	0.12	0.12	0.12	0.12	0.12	0.11	0.11	0.11	0.07
Potassium, %	1.60	1.77	1.74	1.81	1.72	1.57	1.38	1.48	0.65
Sodium, %	0.38	0.61	0.57	0.66	0.55	0.36	0.36	0.46	0.4
Iron, ppm	86.80	106.97	103.88	114.38	101.43	65.34	59.91	91.24	100
Copper, ppm	8.00	11.81	11.22	12.83	10.75	6.27	3.97	8.80	10
Zinc, ppm	92.00	78.76	80.75	78.28	82.33	75.32	80.94	74.71	40
Selenium, ppm	1.10	0.72	0.78	0.67	0.83	0.83[3]	0.85[4]	0.80	0.3
Vitamin E, IU/kg	108.0	198.0	185.0	222.0	174.0	81.0[4]	74.0[4]	162.0	50

[1]VibraLife (Hampel Animal Care, Germantown, WI, 53022)

[2]100% of the formula ingredients have a value for nutrient listed and ingredient ratios are measured on a ‘by weight’ basis; Formula ingredients: **WGM** = Whole Goat’s Milk (Meyenberg, Turlock, Ca, 95381); **DM** = Doe’s Match (Land O’ Lakes Animal Milk Products Co., Shoreview, MN, 55126); **PGM** = Powdered Goat’s Milk (Meyenberg, Turlock, Ca, 95381); **WCM** = Whole Cow’s Milk (Wholesome Farms, Sysco San Diego, Poway, Ca, 92064); **WHEY** = Whey Isolate Powder (The Milky Whey Inc., Missoula, MT, 59801); **H20** = Water (tap).

[3]33% of the formula ingredients have a value for nutrient listed.

[4]67% of the formula ingredients have a value for nutrient listed.

[5]NRC (2001) nutrient concentrations for a 45 kg dairy calves fed milk replacer containing 4.75 kcal ME at rate of 0.53 kg DM (1.0% BW) per day.

Table 3. Nutrient concentrations (DMB) of Vibra-Life[1] vitamin and mineral supplement.

Nutrient	**Guaranteed analysis[2]**	**Commercial laboratory Analysis[3]**
Calcium, %	3.5 -4.5	3.32
Phosphorus, %	0.5 – 0.85	0.83
Ca:P ratio	nr[4]	1.70
Magnesium, %	nr	1.81
Potassium, %	nr	6.39
Sodium, %	nr	0.21
Iron, mg/L	nr	19100.00
Copper, mg/L	nr	952.00
Zinc, mg/L	nr	17700.00
Selenium, mg/L	240.0	na[5]
Vitamin E, IU/kg	22,000	na

[1]Hampel Animal Care, Hamel Corp., Germantown, WI, 53022.
[2]Manufacturer's label.
[3]Dairy One, Ithaca, New York, 14850.
[4]nr = not reported.
[5]na = not analyzed.

Table 4. Serum copper, iron, vitamin E, and selenium concentrations for 20 pre-ruminant species fed milk-replacers containing Vibra-Life vitamin and mineral supplement.

Species	Sample Size	Serum Copper, mg/L		Serum Iron, mg/L		Serum Vitamin E, mg/L		Blood Selenium, mg/L	
		Range[1]	S.D.[2]	Range	S.D.	Range	S.D.	Range	S.D.
Addra Gazelle *(Nanger dama ruficollis)*	1	1.10	--	3.40	--	2.50	--	0.50	-
Armenian Mouflon *(Ovis aries orientalis)*	1	1.30	--	1.90	--	0.96	--	0.36	-
Blackbuck *(Antilope c. cervicapra)*	8	0.74 – 1.50	--	0.54 - 4.00	1.20	0.89 – 4.60[4]	1.60	0.28 – 0.59[4]	0.13
Domestic Cattle *(Bos t. taurus)*	1	0.70	--	1.70	--	na[3]	--	na	--
East African Sitatunga *(Tragelaphus s. spekii)*	1	0.87	--	2.40	--	2.10	--	0.17	--
Eastern Bongo *(Tragelaphus eurycerus)*	2	1.20 – 1.30	0.07	2.70	--	5.20	--	0.41	-
Eastern Giant Eland *(Taurotragus derbianus gigas)*	1	1.80	--	0.92	--	3.10	--	0.15	--
Eastern White Bearded Gnu *(Connochaetes taurinus albojubatus)*	1	0.76	--	2.00	--	1.90	--	0.43	--
Fringe-eared Oryx *(Oryx beisa callotis)*	1	1.30	--	na	--	6.80	--	0.57	--
Grant's Gazelle *(Nanger g. granti)*	2	1.00 – 1.10	0.07	1.50 – 2.80	0.92	2.50 – 2.80	0.21	0.63 – 0.69	0.04
Kenya Impala *(Aepyceros melampus rendilis)*	2	0.99 – 1.30	0.22	1.50 – 3.60	1.48	3.30	--	0.67	--

Table 4 (cont.). Serum copper, iron, vitamin E, and selenium concentrations for 20 pre-ruminant species fed milk-replacers containing Vibra-Life vitamin and mineral supplement.

Species	Sample Size	Serum Copper, mg/L		Serum Iron, mg/L		Serum Vitamin E, mg/L		Blood Selenium, mg/L	
		Range[1]	S.D.[2]	Range	S.D.	Range	S.D.	Range	S.D.
Nubian Ibex *(Capra nubiana)*	1	1.40	--	1.40	--	4.50	--	0.35	--
Scimitar-horned Oryx *(Oryx dammah)*	1	1.10	--	0.64	--	2.10	--	0.38	--
South African Greater Kudu *(Tragelaphus s. strepsiceros)*	2	0.96 – 1.30	0.24	1.40 – 2.50	0.78	2.40 – 2.60	0.14	0.26 – 0.30	0.03
South Africa Sable Antelope *(Hippotragus n. niger)*	1	1.10	--	1.50	--	na	--	na	--
South African Springbok *(Antidorcas m. marsupialis)*	3	0.88 – 2.20	0.58	1.40 – 3.90[5]	1.39	3.20 – 3.80[5]	0.42	0.39 – 0.42[5]	0.02
Southern Gerenuk *(Litocranius w. walleri)*	1	0.41 – 0.72	0.22	2.30 – 2.50	0.14	5.80	--	0.52	--
Thomson's Gazelle *(Eudorcas t. thomsonii)*	6	0.57 – 1.50	0.32	0.87 – 3.80	1.08	1.60 – 5.60[6]	1.45	0.23 – 0.30[6]	0.03
Transcaspian Urial *(Ovis aries arkal)*	2	0.71 – 0.80	0.06	3.90 – 4.20	0.21	0.36 – 2.70	1.65	0.38 – 0.40	0.01
Turkomen Markhor *(Capra falconeri heptneri)*	1	0.74	--	1.40	--	3.80	--	0.43	--
Across all species	**39**	**0.41 – 2.20**	**0.34**	**0.54 – 4.20**	**1.04**	**0.36 – 6.80[7]**	**1.56**	**0.15 – 0.69[7]**	**0.15**

[1]Single value represents result for 1 animal.
[2]S.D. = Standard deviation; cells with hyphens were not calculated.
[3]na = not analyzed.
[4]Values listed represent results for 5 of 8 animals.
[5]Values listed represent results for 2 of 3 animals.
[6]Values listed represent results for 5 of 6 animals; 1 animal had no detectable Vitamin E concentration.
[7]Range value represents 30 of 39 animals.

EVALUATION OF BROWSE COMPOSITION: VITAMIN E

Katherine Kerr[1,2]*, Kathleen Sullivan[1,2], Shannon Livingston[1], Charles Staples[2], and Eduardo V. Valdes[1 - 4]

[1]Disney's Animal Kingdom, Lake Buena Vista, FL 32830 USA
[2]University of Florida, Gainesville, FL 32611 USA
[3]University of Guelph, Ontario, Canada
[4]University of Central Florida, Orlando, FL 32816 USA

Abstract

Vitamin E is an important antioxidant, and deficiency is known to impact the reproduction, growth, and immunity in many species. Dietary browse can be an important source of vitamin E for animals housed in zoological institutions; however the contribution of these items to total dietary vitamin E likely varies by browse species and across time. Our objective was to evaluate this variation utilizing browse harvested at Disney's Animal Kingdom. Samples of select browse species were collected monthly over the course of one year (June 2014 to May 2015) during periods of inclusion within animal diets: whole stalk, including leaves, of elephant grass (*Pennisetum purpureum*; n = 30 samples; 3 samples/mon for 10-mon); leaves of Japanese blueberry (*Elaeocarpus sylvestris;* n = 36 samples; 3 samples/mon for 12-mon); and branches, including leaves, of willow (*Salix caroliniana;* n = 33 samples; 2 to 3 samples/mon for 12-mon). We utilized Covance laboratories for vitamin E analysis (Table 1), and Dairy One laboratories for proximate analysis (Table 2).

Elephant grass stalks (*Pennisetum purpureum*): Vitamin E concentrations (78.8 ± 21.9 mg/kg DM; Range: 0 to 281 mg/kg DM) in elephant grass stalks varied; however they were not significantly different ($P > 0.10$) across time. Mean vitamin E concentrations of elephant grass stalks were the lowest among the species each month, being similar ($P > 0.10$) to willow concentrations only in January, February, and May.

Japanese blueberry leaves (*Elaeocarpus sylvestris*): In general, vitamin E increased steadily from June (464 ± 60 mg/kg DM) to December (1931 ± 69 mg/kg DM), and decreased steadily from December to May (492 ± 69 mg/kg DM). However, in October (457 ± 69 mg/kg DM), vitamin E concentrations were lower than expected, while in April concentrations were (1132 ± 69 mg/kg DM) higher than expected. Mean vitamin E concentrations of Japanese blueberry leaves were the highest among the species each month, being similar ($P > 0.10$) to willow concentrations in June, July, and October.

Willow branches (*Salix caroliniana*): In general, mean Vitamin E concentrations of willow branches were highest between June and November (391 to 577 mg/kg DM) versus December to May (117 to 320 mg/kg DM). Concentrations peaked in September, and were lowest in February. Mean vitamin E concentrations of Willow branches were intermediate among the species each month.

An understanding of vitamin E concentrations over time in browse is important for optimization of dietary formulations for animals housed in zoological institutions. For example, herein we

found that contribution of Elaeocarpus to dietary vitamin E would be more than 4 times higher utilizing data from December compared to data from June. These data highlight the importance of spacing quality control samples for browse species overtime to ensure an adequate representation of dietary vitamin E concentrations are obtained.

Table 1. Vitamin E of three browse species collected monthly over the course of one year (June 2014 to May 2015).

Vitamin E, mg/kg DM	**Japanese Blueberry** *Elaeocarpus sylvestris*	**Elephant Grass** *Pennisetum purpureum*	**Willow** *Salix caroliniana*
LS Mean ± SEM	971.6 ± 20.2	78.8 ± 21.9	348.5 ± 22.3
Range	319.0 to 2319.5	0.0 to 281.3	114.8 to 814.3

Table 2. Nutrient composition of three browse species collected monthly over the course of one year (June 2014 to May 2015).

Nutrient	**Japanese Blueberry** *Elaeocarpus sylvestris*	**Elephant Grass** *Pennisetum purpureum*	**Willow** *Salix caroliniana*
Dry Matter (DM), %	34.2 ± 3.5	22.0 ± 3.5	35.2 ± 5.2
Gross Energy cal/g DM	4670.4 ± 137.0	4515.9 ± 153.1	4978.5 ± 122.0
Crude Protein, % DM	13.2 ± 2.1	9.8 ± 2.3	10.3 ± 1.9
Crude Fat, % DM	3.6 ± 0.4	3.0 ± 0.4	3.0 ± 0.8
ADF, % DM	21.3 ± 4.0	38.3 ± 2.8	38.5 ± 4.8
NDF, % DM	35.3 ± 5.3	68.7 ± 3.6	48.9 ± 5.2
Lignin, % DM	8.7 ± 2.0	3.7 ± 1.1	13.8 ± 1.6
Starch, % DM	6.4 ± 4.4	0.5 ± 0.3	1.8 ± 1.1
Water Soluble Carbohydrate, % DM	20.5 ± 5.3	8.3 ± 1.4	13.2 ± 3.2
Simple Sugars (ESC), % DM	11.5 ± 3.9	6.6 ± 1.9	8.8 ± 2.0

FEEDING FRENZY: TURNING THE SCIENCE OF ZOO NUTRITION INTO A GAME FOR KIDS.

Bethany Fisher* and Heidi Bissell

Busch Gardens Tampa, 3605 E. Bougainvillea, Tampa, FL 33612

Abstract

In 2012, Busch Gardens opened the Animal Care Center to the public to share general information about the techniques and science involved in preventative and emergency care of the 12,000 animal residents of the zoo as well as local injured wildlife. Zoo educators teamed up with the veterinary and nutrition staff to develop demonstrations and interpretive elements that would share advanced scientific principles with a general audience. The concept of using a board game to teach children the basic concepts of zoo nutrition was developed into a working prototype, starting with current nutritional data for common foods (Kerr 2014, Mazuri 2015, Schmidt 2005, USDA 2014) and approximate relative values for animal nutritional requirements. To simplify the numbers for gameplay, nutritional composition was scaled down to integers ≤ 6, while maintaining approximate relative values, and the animals requirements were set at a maximum of 20 “nutritional units”. To ensure playability, the conceit was made that every animal represented in the game could have their nutritional needs met using five food cards, with multiple alternative solutions being possible. The resulting game is used as an interpretive tool by educators to start conversations about the science of nutrition and the interplay between animal husbandry, nutrition, and veterinary care.

Gameplay & Methodology

The game involves the use of “animal boards”, three types of “food cards” (produce, pellet, meat), and three types of “food points” (protein, fat, carbohydrates). Each animal board displays a value for each type of food point needed to win, with each value equal to or less than 20. The boards also indicate which types of food cards the player may use to meet their animal’s needs (referencing whether the animal is a carnivore, omnivore, or herbivore). The food cards are color coded as produce, pellet, or meat, and indicate a given value of the three types of food points. The goal of the game is to be the first player to meet the animal’s requirements exactly.

For the purpose of the game, the value of carbohydrate was crudely estimated as:

Carbohydrates by difference = 100% - (water% + protein% + fat% + ash%)

Values for protein and fat content were derived from the USDA (Schmidt, 2005; USDA, 2014) and manufacturers’ websites (Mazuri, 2015), as well as other resources for whole prey items (Kerr, 2014). All values were then scaled down to values less than six, rounded to the nearest integer.

Animal nutritional requirements were based on relative percentages of fat to protein to carbohydrate, scaled down so that no value exceeded 20. To ensure playability, a combination of five food cards was chosen for each animal based loosely on their diet given at the zoo, which led in some cases to deviation from established values. Some artistic license was also taken to ensure each animal board showed different values, even if the animals had similar requirements on the smaller scale.

Turaco	17	15	68	4	4	17
Solution	16	16	80	4	4	20
106%	94%	107%	118%	100%	100%	118%
MAZURI ZULIFE SOFT-BILL DIET (5M12)	82	78.3	231.45	2	2	6
MAZURI ZULIFE SOFT-BILL DIET (5M12)	82	78.3	231.45	2	2	6
BANANA	9.84	10.305	221.5417	-	-	6
GREENS, MIXED CHOPPED	11.92	5.22	29.07775	-	-	1
APPLE	0.76	3.24	59.0267	-	-	1

Figure 1. Example of one five-card solution (using a pellet card twice), comparing original requirement values and simplified gameplay values.

	Protein	Fat	Carb.
PRODUCE			
Apple	0	0	1
Banana	0	0	6
Green Beans	0	0	1
Corn	0	0	1
Carrot	0	0	1
Grapes	0	0	2
Kale	0	0	1
Onion	0	0	1
Papaya	0	0	1
Pear	0	0	2
Romaine	0	0	1
Squash	0	0	1
PELLETS			
High Fiber Biscuits	2	1	4
Leaf-eater Biscuits	2	1	5
Low Iron Bird Pellets	2	2	6
Parrot Pellets	2	1	6
Primate Biscuits	2	1	4
MEATS			
Boneless Skinless Chicken Breast	3	1	0
Chicks	2	1	0
Ground Large Carnivore Diet	4	5	1
Ground Small Carnivore Diet	2	2	1
Hard-Boiled Egg	2	5	0
Mice	2	2	1
Rabbit	2	1	1
Rat	1	2	0

Figure 2. Summary of food card values used in prototype game.

	Protein	Fat	Carb.
Gorilla†	6	3	16
Hornbill*	9	9	3
Hyena*	16	13	3
Lemur†	2	1	16
Parrot†	2	4	18
Serval*	14	11	3
Sloth	4	6	8
Tiger*	14	9	3
Turaco†	4	4	20

* = can only use meat cards

† = can only use produce & pellet cards

Figure 3. Summary of animal nutritional requirements used in game prototype.

Figure 4. Example of animal board game piece used in working prototype.

Figure 5. Examples of food cards used in working prototype.

Discussion

Although originally designed to cater to children aged 9 and up, the game "Feeding Frenzy" has been very well received by guests of ages from 4 to adult. The carnivores have proven to be more challenging for guests to solve than herbivores, with the most common challenge expressed by guests to be balancing fat with protein intake. Time taken to reach a solution varies widely, with carnivores taking 2-3 times longer to solve than herbivores or omnivores. This divergence has been used to present two difficulty levels of the game to cater towards younger or older guests. The assurance of a five-card solution gives guests confidence that a solution exists, although many guests have discovered their own 3- or 4-card solutions. A surprising number of guests ignore visual clues given on the "pellet" cards indicating animal preferences, which commonly results in the parrot pellets being chosen in large quantity for the primates, and vice versa. Future versions of the game will likely include more omnivores and food choices, including a "browse" produce card that provides protein. The gorilla and sloth boards may also be modified in future versions so that a solution can be reached using the browse card. As an interpretive tool, the game has proven valuable in starting conversations about the interactions between animal husbandry, nutrition, and veterinary medicine, and the important role zoo nutrition plays in animal health and wellbeing.

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THE GROWTH RESPONSES OF TWO POLAR BEAR (*URSUS MARITIMUS*) CUBS TO REGIMENTED DIETARY ENERGY.

Jaap Wensvoort[1]*, Gregory Thiemann[2], Dawn Mihailovic[1], Jim Atkinson[3], and Ian Duncan[3]

[1]Toronto Zoo, 361A Old Finch Avenue, Toronto, Ontario, Canada
[2]York University, 4700 Keele Street, Toronto, Ontario, Canada
[3]University of Guelph, 50 Stone Road East, Guelph, Ontario, Canada

Introduction

The growth of polar bear (*Ursus maritimus*) cubs depends to a large extent on litter size and the provision of maternal care, specifically, milk production and the sharing of prey (Derocher and Stirling, 1996, Robbins et al., 2012). Cubs in most subpopulations stay with their mothers for up to 2 ½ years and rely solely on maternal milk during the first 3 months of life. Subsequently, they consume milk and prey, however, the amounts and proportions vary, particularly since 20% of females with cubs and 60% of females with yearlings will stop lactating at some point during the ice-free summer season (Derocher et al., 1993).

Structural body size in polar bears is variable across subpopulations and is dependent upon nutritional and genetic factors, including gender (Derocher and Stirling, 1996, Derocher and Wiig, 2002). Even within structural body size, the mass of polar bears can vary considerably across years and season due to patterns of hyperphagia and fasting, dictated by environmental conditions and opportunistic feeding behaviors (Knudson, 1978, Atkinson and Ramsay, 1995, Stirling, 2011, Derocher and Lynch, 2012). Adult female polar bear mass may change up to four-fold within a year (Stirling, 2011, Derocher and Lynch, 2013). Variation in body mass relative to nutrition of growing polar bear cubs is not well understood.

Materials & Methods

In 2011 and 2013, polar bear cubs were born at The Toronto Zoo and in each year only one male cub survived. Both cubs were removed from their mothers and hand reared within the zoo's Wildlife Health Centre (Mihailovic et al., 2012). After 16 and 14 weeks, respectively, the cubs were transferred to the polar bear house maternal unit, which included indoor and outdoor holding areas, a small pool, and weighing scales. Cubs were kept on a regimented diet of known composition until almost one year of age. The controlled feeding regime provided a unique opportunity to document the growth of polar bear cubs as a function of dietary energy.

This diet was composed of increasing amounts of Toronto Zoo Feline Diet, dog chow, fish oil and smelt, with a consistent amount of milk replacer. It was decided to let the cubs develop gradually and to adjust their daily feed supply (aiming at maximum 5 % increase per week) according to their weekly gains in weight. The cubs were fed several times per day and the majority (estimated over 99%) of the diet was consumed, and so an assumption was made of 100% intake. The cubs were weighed once a week.

The metabolizable energy (ME) content of the diet was estimated by using equations derived for dogs (National Research Council, 2006), or was obtained from product diet labels. Preliminary calculations are presented in Table 1, and Figures 1 and 2.

This work was done to support conservation of polar bears in the wild.

Table 1: Total and daily growth and metabolizable energy (ME) intake and daily energy supply related to maintenance and growth of two polar bear cubs on a regimented diet.

Polar Bear	Total ME (MJ)*	Total Growth (kg)	Daily ME Intake (MJ/day)*	Daily Growth (kg/day)	ME /kg Growth (MJ)*
Cub 1	6122	100.2	25.8	0.42	61
Cub 2	5863	79.3	24.3	0.33	74

*Please note: These amounts of ME are for maintenance, growth and movement for a polar bear cub reared in human care only.

**Please note: During the summer of 2012, for a period of 20 days, ethograms were made during mornings and afternoons of the 2011 cub. This was performed in anticipation of behavioral changes due to potential heat stress. No clear signs of behavior adaptations to excessive heat (like decreased activity, staying in the shade, panting, etc.) were observed.

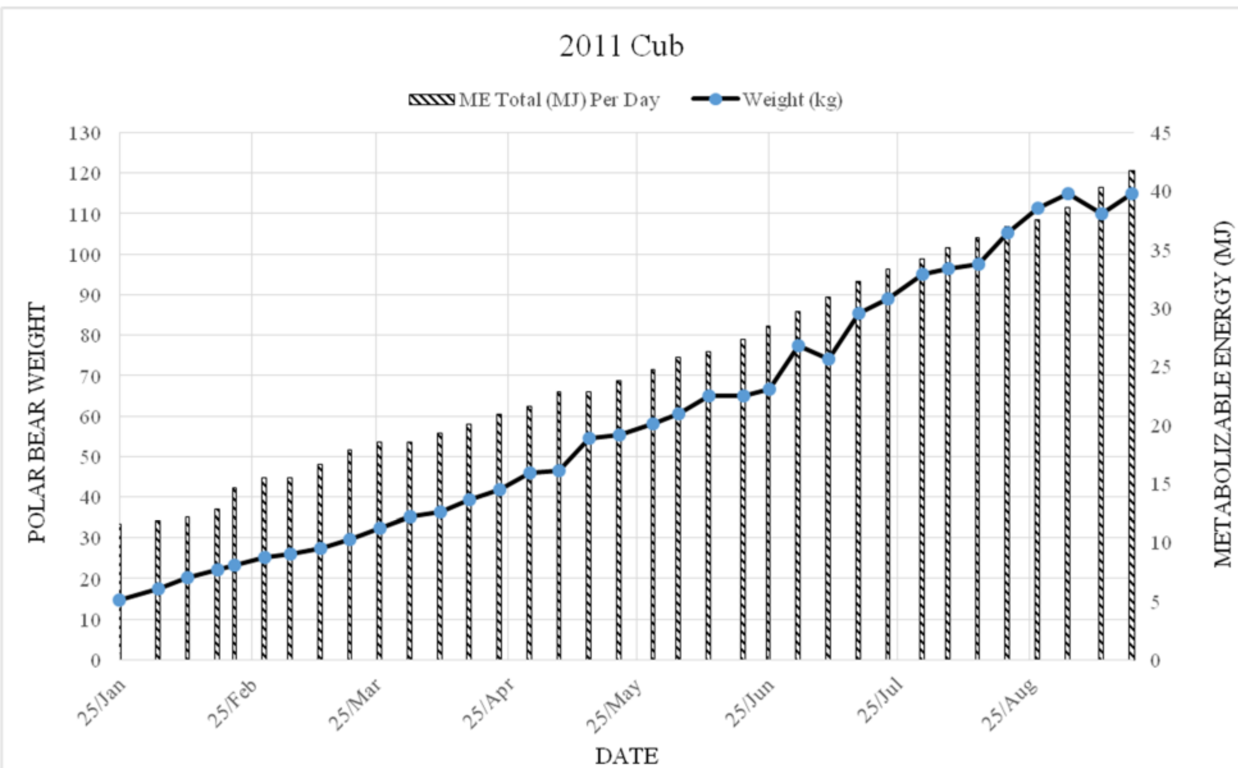

Figure 1. Cub weight and daily ME intake at week intervals, January to September 2012

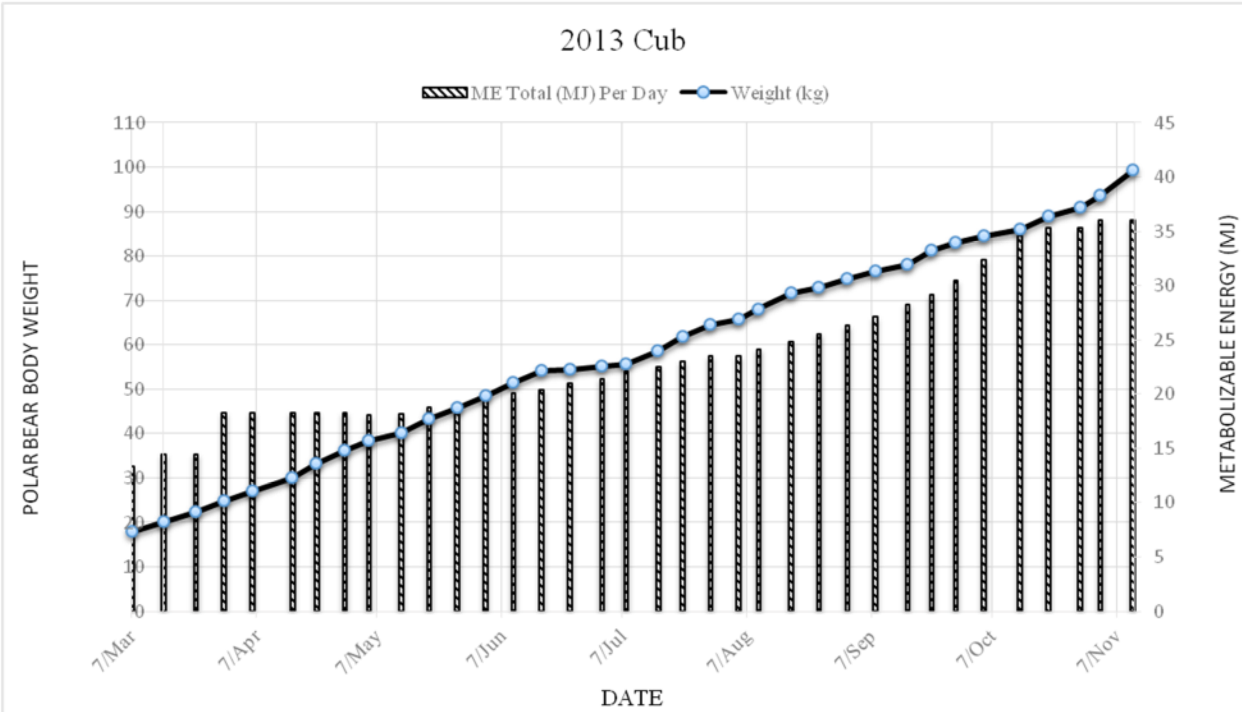

Figure 2. Cub weight and daily ME intake at week intervals, March to November 2014

Acknowledgments

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HAND-REARING AND GROWTH OF A GREY SEAL (*HALICHOERUS GRYPUS*)

Erin Kendrick[1]*, Michael Maslanka[1], and Rebecca Sturniolo[2]

[1]Smithsonian Conservation Biology Institute, National Zoological Park, Center for Animal Care Sciences, Department of Nutrition Science, 3001 Connecticut Ave., Washington, DC, 20008, USA
[2]Smithsonian Conservation Biology Institute, National Zoological Park, Center for Animal Care Sciences, American Trail, 3001 Connecticut Ave., Washington, DC, 20008, USA

Abstract
Typical grey seal lactation lasts 15-21 days, followed by abrupt weaning by the dam and a several weeks long period of relatively significant weight loss while the pup learns to feed and fend for itself. It was with this natural history in mind that we formulated a grey seal hand-rearing protocol pending the birth of a female pup in early 2014. After the pup showed continuous weight loss, despite frequent nursing bouts, we elected to assist-feed. The pup was pulled for scheduled tube-feedings every day, but otherwise remained with the dam for rearing up until weaning. Through the hand-rearing and weaning process, she continually defied expectations in terms of her consumption, activity level, and weight gain. Weight gains were slow in the beginning, leading to questions about energy consumption vs. expenditure. When she obtained an appropriate weight for weaning she did not experience the period of expected weight loss while learning to eat fish, leading to excess body condition during the first year of growth. While our efforts were ultimately successful, these experiences led to alterations of our hand-rearing protocol.

IMPACT OF DIETARY N-3 AND N-6 PUFA ON OXIDATIVE STATUS AND INFLAMMATION IN YELLOW-RUMPED WARBLERS

Morag F Dick[1]*, Laura A Rooney[1], & Christopher G Guglielmo[1]

[1]Department of Biology, Advanced Facility for Avian Research, University of Western Ontario, Canada

Abstract

The intake and ratio of the essential fatty acid classes, n-3 and n-6 polyunsaturated fatty acids (PUFA), impact animal health. The dietary ratio of these fatty acids can be important as n-3 PUFA are considered anti-inflammatory, while n-6 PUFA are pro-inflammatory. Additionally, the increased number of double bonds in PUFA makes them more vulnerable to oxidative damage, potentially increasing the demand for antioxidants. Wild passerine diets differ greatly in intake and ratio of PUFA, with the ratios often beyond what is generally considered healthy for humans and domestic species. Limited data is available on the effect of n-3 and n-6 PUFA on inflammation and oxidative stress in exotic bird species. We studied the influence of n-3 and n-6 PUFA intake on the health of yellow-rumped warblers (*Steophaga coronata*). Warblers were fed diets enriched in either n-3 or n-6 PUFA, or a low PUFA control diet for 6 weeks and then sampled at rest or after an endurance flight in a wind tunnel. Plasma haptoglobin was measured as an indicator of general inflammation in the body, and neither diet nor flight influences its values. Further analyses are on-going but will include the antioxidant capacity and markers of oxidative damage in the liver and flight muscle. Fatty acid analysis of the plasma, liver and muscle will also be completed. The influence of dietary PUFA on oxidative capacity and damage will be reported and related to the fatty acid composition of the tissues. This study will help further our understanding of PUFA nutrition by directly testing for potential negative effects of n-3 and n-6 PUFA, and can be used in the formulation and evaluation of captive passerine diets.

INTERPRETING VITAMINS AND MINERAL CONCENTRATIONS IN SERUM OF EXOTIC SPECIES: LAB VALUES ARE NOT INFALLIBLE

Kathleen E. Sullivan[1,2], Shannon Livingston[1], Katherine Kerr[1,2], and Eduardo V. Valdes[1-4]*

[1]Disney's Animal Kingdom, Lake Buena Vista, FL 32830 USA
[2]University of Florida, Gainesville, FL 32611 USA
[3]University of Guelph, Ontario, Canada
[4]University of Central Florida, Orlando, FL 32816 USA

Abstract

The prospect of trust and laboratory analysis is often so frightening a concept, scientists in the zoo field, including nutritionists and veterinarians, do not discuss it as more than a side note. Often we trust that specialists in biochemistry and new analysis technologies ensure proper verification of lab methodology, and would inform the consumer of any difficulties or questionable data. In a field where species' serum normals are generally not well established, serum data from animals under human care can be one of the major indicators used to judge health. Supplementation plans are developed and medical actions taken, based on serum indices-generally taken as fact and at face value as truth. Correct interpretation of nutritional serum parameters is one layer of possible error. However, commercial laboratories are not without human and instrumentation error as well. Use of equipment such as inductively coupled plasma emission spectrometer (ICP-ES) for mineral analysis, and high performance liquid chromatography (HPLC) for vitamin analysis need not only automated systems, but skilled technicians. Communication regarding samples (frozen, level of hemolysis, use of dilution, etc.), and general attention to detail in results is paramount for animal caretakers to interpret results accurately. Interpreting samples requires knowledge of best method of analysis, and factors affecting meaning – such as whole red blood cell manganese being the best indicator of Mn levels. Therefore, while lab error may be a consideration, other factors such as hemolysis may be affecting the wide range of seemingly improbably high levels observed (normal levels of Mn in cattle: 5.0 – 6.0 ng/ ml; sheep: 1.8 – 2.0 ng/ml; pig: 3.0 – 4.0; (Underwood and Suttle, 1999); horse: ~6.0 (Puls, 1994; Figure 1 and 2).

Any academic and scientifically rigorous study requires controls – often both positive and negative controls, as indicators that assays remain within specific coefficients of variance and tell an accurate story – that repeated measures can be trusted. In exotic animal health, controls are often forgotten, but they can be easily tested. This can be done with a lab standard or a large blood draw where a repeat sample is sent with each batch. In a preliminary set of elephant serum samples sent to two different commercial laboratories for vitamin E analysis, with each sample split from the same sampling tube after centrifugation, some startling differences could be seen (Table 1).

While often taken for granted, laboratories that we invest in for analysis will be open and honest with their methodologies, communicate regarding irregular samples, and discuss the issues regarding retests, and often the samples themselves. When serum values received affect treatment plans and dietary supplementation, it is well worth the time and scrutiny to evaluate all factors, including the reliability of the laboratory. It is difficult for professionals to make

decisions about proper treatment (e.g. supplementing or not) for animals under their care when questions remain regarding the validity of results obtained.

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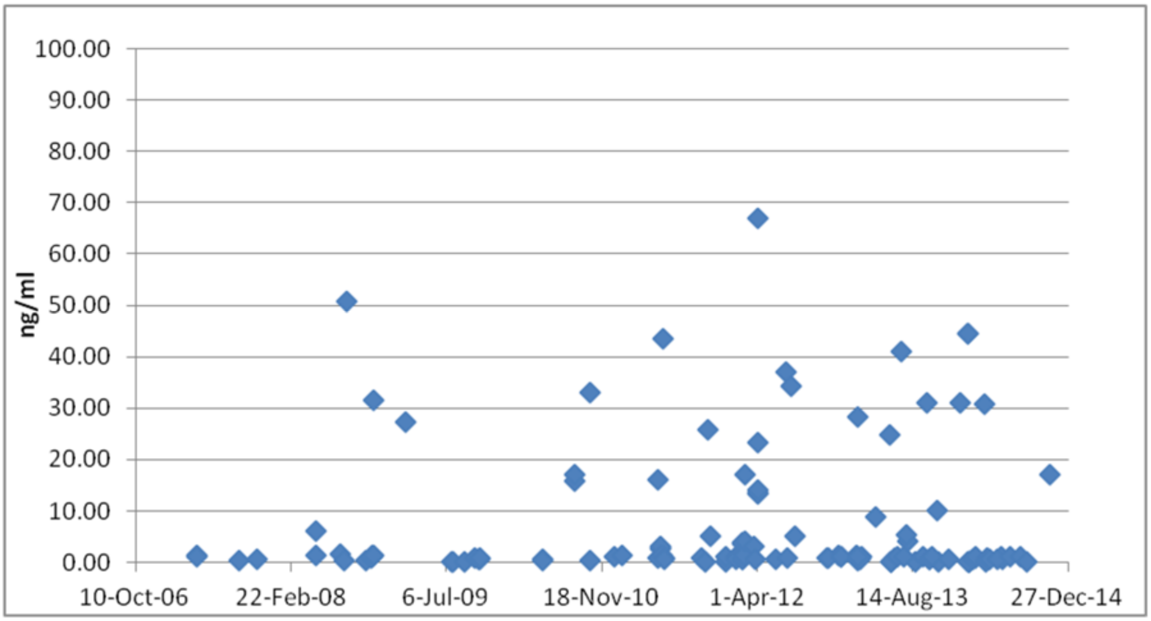

Figure 1. Serum Mn (ng/ml) in Zebra species across 8 years at Disney's Animal Kingdom.

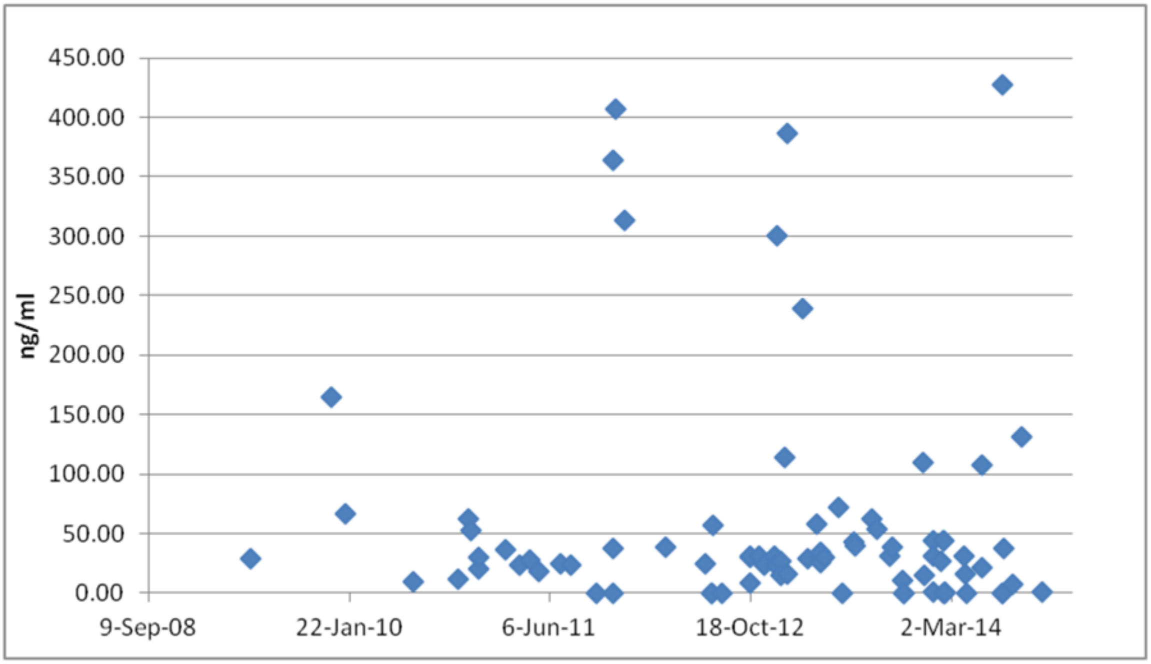

Figure 2. Serum Manganese (ng/ml) across multiple species over 6 years at Disney's Animal Kingdom.

Table 1. African Elephant Vitamin E values on the same samples sent to 2 commercial laboratories in 2015 from Disney's Animal Kingdom

	Vitamin E (ug/ml) Lab 1	**Vitamin E (ug/ml) Lab 2**	**Difference (Lab2 minus Lab 1 values)**
Average ± SEM	0.47 ± 0.07	0.74 ± 0.08	0.27 ± 0.06*
Minimum	0.18	0.32	-0.09
Maximum	0.87	1.09	0.49
N	10	10	10

*$P < 0.05$ between Lab 1 and Lab 2

INVESTIGATION OF A NEW DIET FORMULA FOR CAPTIVE SHORT-BEAKED ECHIDNAS (*TACHYGLOSSUS ACULEATUS)*

Hayley J. Stannard[1,4], Jamie M. Bekkers[2], and Michelle Shaw[3]*

[1]Water and Wildlife Ecology Research Group, School of Science and Health, University of Western Sydney, New South Wales (NSW) 2751, Australia
[2] The Van Hall Institute, Postbus 1528, Leeuwarden, Netherlands
[3]Taronga Conservation Society Australia, Bradleys Head Road, Mosman NSW 2088, Australia
[4] Current address: School of Biological Sciences, Charles Perkins Institute, University of Sydney, Sydney NSW 2000, Australia.

Abstract
Short beaked echidnas (*Tachyglossus aculeatus*) are myrmecophages, ant and termite specialists, and replicating their diet in captivity is problematic. Captive diets often incorporate raw meat, eggs and cat food mixed together with water and vitamin and mineral supplements. These diets have presented a number of health problems, such as cystitis, gut impaction, obesity and diarrhea. This study aimed to assess the acceptability and palatability of a manufactured diet designed for insectivores. Compared to the previous diet, the new diet provided higher levels of carbohydrates, lower lipids and similar levels of energy and protein. The new diet was readily accepted by the echidnas. Time spent feeding averaged 4 minutes per 100g test diet vs. 5.6 minutes per 100g original diet. Daily digestible energy intake was 250 kJ $kg^{-0.75}$ d^{-1}, which is within the range of daily energy expenditure values previously published. Digestibility values were above 74% for both diets for all nutrients studied. Generally, digestibility values were lower for most nutrients on the new diet due to the higher portion of indigestible plant material in that diet. Faecal consistency was ideal (averaged 2.3/5.0) on Taronga's original diets and the test diet. Following the study, all twenty-four short beaked echidnas and two long-beaked echidnas at Taronga Zoo and Taronga Western Plains Zoo were transitioned onto the new diet and have been maintained successfully for over eight months. The new diet was determined to be acceptable for maintaining echidnas in captivity.

MANAGING DIABETES THROUGH DIET IN BLACK-FOOTED TREE RATS (*MESEMBRIOMYS GOULDII)* AND GREATER STICK-NEST RATS (*LEPORILLUS CONDITOR*)

Corinne Lendon[1], Frances Hulst[2], and Michelle Shaw [2]*

[1]School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch WA 6150, Australia.
[2]Taronga Conservation Society Australia, Bradleys Head Road, Mosman New South Wales (NSW) 2088, Australia.

Abstract
Clinical and pathological signs consistent with diabetes mellitus (DM) were observed in a collection of Black Footed Tree Rats (BFTR; *Mesembriomys gouldii*) and Greater Stick-Nest Rats (GSNR; *Leporillus conditor*) housed at Taronga zoo. Review of the medical records of eighteen BFTR between 1991 and 2014, and thirty eight GSNR between 1995 and 2014, revealed a high frequency of animals with cataracts, obesity, hyperglycemia and glucosuria. A diagnosis of DM was made in 78% of BFTR and an estimated 55% of eleven GSNR which had sufficient data for assessment. Obesity is a well-established risk factor for T2DM across species. Initial conservative attempts to reduce weight by lowering energy density were unsuccessful in these rodents. A review of the literature suggested that T2DM in BFTR and GSNR was similar to several related South American and North African rodents also prone to T2DM when fed a relatively high energy rodent pellet. Intervention by feeding a closer approximation to the native diets had alleviated, even prevented signs of T2DM in some of the reviewed species. The BFTR and GSNR at Taronga zoo were put on a strict low glycemic diet similar to the nutrient composition of their low carbohydrate, high fibre native diets. Significant reductions in body weight, blood and urine glucose resulted. After two months on the diet, 89% of all cases became normoglycemic. The results of this dietary intervention support the initial diagnosis of T2DM and provide evidence for dietary treatment to manage DM in these species.

NUTRITIONAL ANALYSIS OF MIXED PRODUCE FOR EXOTIC SPECIES USING NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS)

Scott Williams[1]*, Kathleen Sullivan[1, 2], Shannon Livingston[1], Katherine Kerr[1, 2], and Eduardo V. Valdes[1-4]

[1]Disney's Animal Kingdom, Lake Buena Vista, FL 32830 USA
[2]University of Florida, Gainesville, FL 32611 USA
[3]University of Guelph, Ontario, Canada
[4]University of Central Florida, Orlando, FL 32816 USA.

Abstract

Near Infrared Reflectance Spectroscopy (NIRS) has long been established as a fast, easy, and cost effective technology for measuring basic organic components utilized in diet formulations. In zoological facilities, accurate timely quality control of diet ingredients, including highly variable items and those with a short shelf life, such as produce, can be difficult. We utilized traditional wet chemistry data from produce items (n = 85) to create novel calibrations using NIRS to measure protein, fat, and carbohydrate composition. This calibration was cross validated and demonstrated strong predictive power for fibers, sugars, protein and dry matter. Further samples across time and season will strengthen this calibration, improving efficacy of frequent quality control.

Introduction

Nutritional profiles of produce vary due to both pre- and post-harvest factors, including cultivar, seasonality, maturity, handling, and storage (Kader, 2001). Capturing this variability in nutrient content for diet evaluation is often challenging in terms of practical costs for frequent wet chemistry analysis and lack of predictability across time and season. The USDA database nutrient information for produce items are average values that may not represent the quality of specific items received at every facility. Ongoing quality control is an important aspect of any animal nutrition program. The ability to utilize Near Infrared Reflectance Spectroscopy (NIRS) to monitor these fluctuations and provide more immediate, cost effective feedback and would allow for better understanding of changing nutritional factors presented to our animals.

Materials and Methods

Our laboratory has sampled produce items (n = 85) as part of an ongoing quality control and analysis program at Disney's Animal Kingdom. Produce items used in animal diets include greens, fruits, vegetables, nuts, and seeds (Table 1). Samples were analyzed at Dairy One Forage Laboratory (Ithaca, NY) for dry matter (DM), crude protein (CP), crude fat, neutral detergent fiber (NDF), acid detergent fiber (ADF), starch, and ethanol soluble carbohydrates (sugar). These wet chemistry data were utilized as reference values for subsequent NIRS calibration development and cross validation (Table 2). The dried and ground samples were returned from Dairy One and then scanned using NIRS (SpectraStar 2500X- RTW System with InfoStar Software v. 3.11.1, Unity Scientific, Brookfield, CT) with reflectance spectra collected over a range of 680-2500nm with 1nm wavelength increments. Calibration development was achieved by treating the full spectra with partial least squares and cross validation of the prediction equation was achieved by dividing the calibration set into groups and predicting nutrient values of every sample in the calibration set.

After initial prediction calibration was performed our results indicated that seed and nut type samples represented a unique subset of samples that would benefit from independent calibration development. For the remainder of the abstract "full set" (n = 69) refers to all samples once these items were removed from the population.

Results and Discussion

The prediction calibration utilizing the full set of samples produced strong R^2 values (0.74 to 0.96). For all constituents tested, R^2 for the cross validation test (R^2CV) was also considered strong (R^2CV= 0.72 to 0.87), except for fat (R^2CV=0.50). For the full set of samples, the calibration standard error (SEC) ranged from 6.9 for sugar to 0.98 for fat. Most of this variability can be attributed to the range in standard deviation (SD) of the wet laboratory value for each constituent. Likewise, SE of cross validation (SECV) showed similar fluctuation from 8.95 for sugar to 1.3 for fat. This is not surprising, considering the range of produce included (dark leafy greens, fruits, and vegetables) with similarly low fat content with highly variable sugars. For the purposes of this calibration, both the SEC and SECV are very acceptable and indicate a calibration with good predictive power. While we continue to collect and add samples across time and season, the calibration predictions can be further improved as unique spectra are identified. The goal is with time to develop specific calibrations to more accurately predict specific items or categories (e.g. leafy greens, high sugar fruits, high fiber vegetables, or apples, sweet potatoes etc.). By consistently scanning and predicting new samples, we can identify those samples which present unique spectra. Incorporating this diversity with wet chemistry data would increase our calibrations predictive robustness; with our aim toward minimize future wet chemistry needs and maximizing the sustained utilization of NIRS technology.

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Table 1. Produce Analyzed for Nutritional Content

Almond	Green Leaf Lettuce	Pineapple
Apple	Green Pepper	Plantain
Banana	Honeydew	Prune
Beet	Kale	Radish
Brazil Nut	Kiwi	Red Kidney Beans
Cantaloupe	Macadamia Nut	Romaine Lettuce
Carrot	Mango	Safflower seed
Coconut	Millet	Strawberry
Corn on the Cob	Napa Cabbage	Sweet potato
Endive	Onion	Tomato
Fig	Orange	Turnip
Frozen Corn	Papaya	Walnut
Garlic	Peanut	White Potato
Grapefruit	Pear	Yellow Squash
Green bean	Pea	Zucchini

Table 2. Laboratory Values for Dried Mixed Produce Samples

Produce, Dry	**NDF**	**ADF**	**Sugar**	**CP**	**Fat**
N	69	69	69	69	69
Min	3.30	1.10	1.50	2.30	0.20
Max	54.70	37.60	80.70	29.00	8.90
Mean	17.87	13.22	33.60	10.98	2.58
SD	12.25	8.05	20.42	7.33	1.93

Table 3. Calibration Statistics for Prediction of Dried Mixed Produce

Produce, Dry	**NDF**	**ADF**	**Sugar**	**CP**	**Fat**
SEC	4.19	2.99	6.90	1.49	0.98
R^2	0.88	0.86	0.89	0.96	0.74
SECV	5.90	4.00	8.95	2.38	1.30
R^2CV	0.73	0.72	0.77	0.87	0.50

PRELIMINARY INVESTIGATIONS INTO CIRCULATING VITAMIN E CONCENTRATIONS IN CAPTIVE OKAPI (*OKAPIA JOHNSTONI*)

Kerri Slifka, MS* and Janis Raines, DVM

Dallas Zoo, 650 S. RL Thornton FWY, Dallas, TX

Abstract

Normal circulating vitamin E levels have not been established for okapi (*Okapi johnstoni*) on a captive diet. Blood samples were obtained in 1996 by the Okapi SSP for free-ranging okapi in Zaire (Lukas, 1996). In 2009, vitamin E levels were acquired for animals housed at the Epulu Station in the Democratic Republic of the Congo fed an all leaf diet without supplementation (Citino, pers. comm). Due to health concerns reported in the Okapi SSP populations regarding circulating vitamin E levels (Petric, 2011), data were compared to levels from Zaire and Epulu. Upon comparison several SSP okapi appeared to have circulating levels 50% lower than previously collected data from Zaire and Epulu. Historically there have been incidences of okapi showing symptoms vitamin E deficiency such as congestive heart failure when pregnant, while other animals have died with congestive heart failure listed as a contributor to mortality. A diet survey conducted in 2011 did not reveal any diets offered to be low in vitamin E compared to probable requirements (Lintzenich and Ward, 1997). The Okapi SSP nutrition and veterinary advisors have requested both banked samples and opportunistic collection from all animals to establish whether circulating vitamin E levels are indeed low in captive US okapi and if there are links to health concerns.

Samples analyzed thus far are presented in Table 1. Results from 75% of submitting institutions had mean vitamin E values greater than or equal to mean values from Epulu or Zaire, however there is substantial individual variation. Six of 11 submitting institution have a minimum values lower than that of the minimum value at Epulu. These preliminary results illustrate the variation in circulating vitamin E concentrations in the captive okapi population. Ultimately a database will be established with vitamin E concentrations for the entire US population. It is hoped that examination of new and previously collected samples will allow comparisons in relation to health status, age, gender and effects of supplementation/diet and establish normal circulating vitamin E concentrations for captive okapi.

Acknowledgements

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Table 1. Serum Vitamin E concentrations in okapi (*Okapia johnstoni*)

Institution	***N***	**Mean (μg/ml)**	**Std Dev**	**Max (μg/ml)**	**Min (μg/ml)**
A	1	0.52	---	---	---
B	1	0.43	---	---	---
C	1	0.10	---	---	---
D	4	0.32	0.15	0.43	0.12
E	15	0.30	0.28	0.88	0.07
F	2	0.13	0.02	0.14	0.11
G	13	0.80	0.50	1.79	0.43
H	5	0.17	0.10	0.31	0.05
I	2	0.78	1.0	1.49	0.07
J	9	0.47	0.35	1.3	0.15
K	6	0.58	0.31	1.02	0.34
Epulu (semi-captive)	12	0.31	0.13	0.57	0.14
Zaire (Free-ranging)	3	0.38	0.17	NA	NA

SO YOU (DON'T) THINK YOU NEED A NUTRITIONIST?

Mike Maslanka, MS[1], Barbara Henry, MS[2], and Ann Ward, MS[3]*

[1]Smithsonian Conservation Biology Institute, National Zoological Park, Center for Animal Care Sciences, Department of Nutrition Science, 3001 Connecticut Ave., Washington, DC 20008, USA
[2]Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220, USA
[3]Fort Worth Zoo, 1989 Colonial Parkway, Fort Worth, TX 76110, USA

Introduction

The first zoo nutritionist was hired at the Smithsonian National Zoo in 1978 (Crissey, 2001a). At the time, this zoo and others were acknowledging the importance of a qualified zoo nutritionist and the value of a zoo nutrition program to the health and welfare of the animals in their collection. To date, nearly 20 AZA accredited facilities in North America, and several others world-wide, have followed suit. However, even given the clear value of a qualified professional on staff to focus their attention on the nutritional care and welfare of the collection, most AZA accredited zoos have not hired full time nutritionists. In lieu of a full time nutritionist, many zoos rely on either their veterinarians, curators, and other animal care staff, or consultants for this pivotal responsibility. There are likely a wide variety of reasons for this, but most may revolve around available resources (primarily financial), an over-valuing of a part-time or consulting focus on collection animal nutrition, and a limited understanding of the benefits of a qualified nutritionist dedicated to the zoological collection. This brief paper addresses some of these issues, and provides background for zoos to justify the hiring and maintenance of a qualified zoo nutritionist as part of their animal care team to meet the mission of their institution and build capacity.

Brief history of Zoo Nutrition in North America

An excellent summary of the history of zoo nutrition was provided by Crissey (2001). Briefly, the discipline of zoo nutrition was initiated in 1918 by Dr. Ellen Corsen-White at the University of Pennsylvania Medical School, who initially studied metabolic bone disease at the Philadelphia Zoological Garden and introduced the recipe for "Zoo Cake" (an uncooked composite ration containing minerals and vitamins).

"*My experience as Prosector to the [Philadelphia] Zoological Society convinced me that during the first six months of the existence of the Garden, the principle causes of death were three: First, improper food, both in quantity and quality; Second, effects of temperature; Third, ill-constructed cages*…." H.C. Chapman, M.D., 1876

Dr. Herbert Ratcliffe, a pathologist at the Philadelphia Zoological Garden, outlined the use of Zoo Cake to develop controlled diets for collection animals that remained in place from the mid 1930's until the late 1970's (Amand, 1980). Ratcliffe reported the development and success of the formulated diets in a clear statement that remains applicable to date: "My aim has been to design diets at reasonable costs that are readily acceptable, that prevent nutritional disease, and that allow animals to develop and maintain high levels of resistance to many of the common agents of disease. Current knowledge of nutrition indicates this may be accomplished by a number of equally satisfactory formulations."

In 1974, the Metro Toronto Zoo hired the first professional commissary manager (Sergio Oyarzun), who developed the position into that of a nutritionist over the years. In the United States, the Smithsonian National Zoological Park hired the first official zoo nutritionist (Olav Oftedal) in 1978, followed shortly thereafter by the creation of a zoo nutrition program at the Chicago Zoological Society's Brookfield Zoo in 1980. The Philadelphia Zoological Garden (1984) and the Wildlife Conservation Society (1986) followed by hiring nutritionists and developing programs. All of these programs grew through the decade(s) after their inception, and other zoos followed suit through the turn of the century. Of note, a zoo nutrition residency program was initially established by Dr. Susan Crissey at the Brookfield Zoo in 1991, which produced three trained nutritionists in place at AZA zoos (currently, Fort Worth Zoo, Cincinnati Zoo & Botanical Gardens, and Smithsonian National Zoo and Conservation Biology Institute). After Dr. Crissey's death in 2002, a residency was established in her honor (Sue Crissey Animal Nutrition Residency Fund), to train future zoo nutritionists, which has generated two zoo nutritionists to date. At the date of this publication, nearly 20 AZA zoos in North America employ at least one trained zoo nutritionist to attend to the nutritional needs of their collections. This represents less than 10% of all accredited AZA zoos in North America.

The field of zoo nutrition began with the documentation of nutritional disease (metabolic bone disease; Amand, 1980) evidenced by pathology. Over the years some employment of nutritionists occurred with more evidence of pathologies related to nutrition – vitamin E deficiency, iron storage disease, metabolic bone disease (again), and vitamin A deficiency, to name a few. Without employment of a pathologist or significant funds dedicated to diagnostic laboratory work, diseases of nutritional etiology may not be accurately diagnosed. Additionally, most institutions do not have dedicated staff, expertise, tools, and/or detailed records to indicate the positive or negative impact of historical diets on the collection. The effects of inadequate/inappropriate feeding can be profound and obvious, but are more often subtle - poor reproduction (or complete lack thereof), poor growth, failure to thrive, predisposition to disease, etc. The root cause of such non-specific "symptoms" is often difficult to discern, at best. The continued growth and development of zoo nutrition as a field, and the increased employment of qualified zoo nutritionists by AZA facilities, will continue to enhance our ability to link these disorders (subtle and otherwise) to their root causes, and provide solutions for the good of animal care, health, and welfare.

Utility spectrum of a Zoo Nutritionist – What can they do for you?

A qualified nutritionist is imperative to the appropriate care and welfare of the zoo animal collection. Among other benefits this individual can bring to the institution are:

- diet evaluation and formulation
- commissary management (inventory and budget management)
- education
- research
- engagement with the professional community via AZA programs
- outreach

Diet evaluation and formulation.

The positive impact on animal care through the formulation and constant evaluation/re-evaluation of diets is difficult to quantify. However, the role of appropriate nutrition was recognized by H.C. Chapman in 1876, and has not fundamentally changed since that time (if anything, its role has been enhanced). Diet formulation is a complex process best described as a matrix of health, requirements, consumption, and management (Crissey, 2001b). Requests for the "ideal" diet for a species or recommendations that can meet the needs of all individuals are unrealistic. Diet formulation is continuous – a change in any aspect of the matrix can cause a cascade of changes throughout, all ultimately impacting the diet formulation. In addition, most collections are dynamic, as well. An investment in a nutritionist is capacity building in that it allows dealing with the needs of enriching and training the collection, changes in the composition of the collection, and unexpected challenges such as hand rearing and critical care. The dynamic nature of the diet evaluation and formulation process challenges the efficacy of consultants (often singular diet review and no provision of implementation, follow-up, or necessary continued re-formulation based on current conditions). The development of diet formulation software and record keeping systems that allow for nutritional data to be maintained and utilized for collection management decisions, and someone to maintain such systems, is, and will be, paramount for providing metrics as a testament to the value of a zoo nutritionist on staff. Though significant developments have been made in the area of computer software, the data generated requires expertise to interpret and apply appropriately. Due to the complexity and ongoing nature of this task, it is best not left to someone on a part time or sporadic basis (staff or consultant), but rather a dedicated and qualified nutritionist able to assess and implement appropriate changes. Critical review of literature and common practices (both peer-reviewed and popular press sources; Ullrey, 1996), knowledge of interrelationships among nutrients, non-nutritive components, and attention to variation in feeds or formulas, among many other things, is essential (Oftedal and Allen, 1996). A qualified nutritionist is trained to consider these aspects and many more to ensure appropriate diets are formulated and evaluated continuously over time. Proactive management, quick response to changing needs, and attention to detail requires significant time and focus, warranting a full time position, rather than part time focus or sporadic attention.

Commissary management (inventory and budget management)

The role of a qualified zoo nutritionist goes beyond diet formulation and evaluation. Having a qualified individual to provide a consistent link between appropriate diets and the provision of those diets is crucial. Often the largest part of any zoo's budget is the food bill, and a qualified nutritionist responsible for not only diet formulation, but also budgeting, allows for fiscal efficiency (Maslanka, 2015). Capacity building and long-term planning includes not only pricing current diets, but also forecasting and providing input on cost of feeding additional/new animals in collection plans and appropriate building of food handling/storage areas in future exhibits. A qualified individual with a solid nutrition background, simply focused on diet ingredient waste (inventory overruns, acceptance of poor quality food items that spoil quickly, overfeeding animals/exhibits, etc) can often initially save a zoo more than their annual salary by focusing on the budget of the operation, efficiency, and waste minimization. Even in well monitored programs "diet drift" occurs over time, and constant assessment over time minimizes its likelihood and impact. Additionally, a nutritionist can set nutrient specifications, knowledgably negotiate feed contracts, critically evaluate feed manufacturers, establish quality control programs, and adjust feeds/ingredients based on availability, sustainability, and cost. Expertise is also required to critically review and select laboratories for nutritional analyses including appropriate laboratory methods and costs. Significant time is needed to critically evaluate food product and industry trends for appropriateness and impact on the health of the collection. A nutritionist has the expertise to oversee biosecurity, food safety/sanitation and the monitoring required for diet ingredients. It is critical to remain up-to-date on product recalls, as well as establish and maintain a food tracking program which allows an appropriate and quick determination of the effect of a recalled product. This may include determining appropriate substitutions, addressing the issue within the supply chain, and follow up with animal care staff regarding how to assess and mitigate the impact of a potentially dangerous food item.

Education

From K-12 programs to local college and university classes/courses, a qualified nutritionist can augment the more "formal" aspects of a zoo education program. Such engagement allows for the dissemination of knowledge and the zoo's mission to a wider audience than just those who come through the gate. It also can clearly define the zoo's commitment to quality animal care and welfare simply by their presence on staff. A zoo with a nutritionist and/or a formal nutrition program can easily contribute to the development of the field through the establishment of volunteer, undergraduate, graduate and post graduate opportunities. Partnerships with local universities can not only expose students to the field of zoo nutrition but also provide students for data collection and research.

Research

Annually, zoos are charged with engaging in conservation research as part of their role within AZA. The field of zoo nutrition remains very much in its infancy in terms of research programs and projects that can positively impact the health and welfare of both zoo and free-ranging animals. The ability of a qualified zoo nutritionist to develop, conduct, and publish research, whether small or large scale, basic or applied, results in an improvement in the health of the collection animals, provides additional notoriety to the zoo itself, as well as further expands the field of zoo nutrition as a whole. Often institutions have current long running field research projects involving animal management and/or animal health staff. A nutritionist can exist as a focus, in and of itself, or it can complement other disciplines and enhance these projects through participation in grant writing, overall guidance, and active participation. Such collaborations increase not only the strength of the existing project, but enhance the knowledge of feeding and nutrition.

Engagement with the professional community via AZA programs

The Association of Zoos and Aquariums is our professional society. As members of that society (as well as, hopefully the Nutrition Advisory Group – the nutrition SAG within AZA), it is each zoos role to contribute when and as they can to the professional goals of AZA. A qualified zoo nutritionist is able to do this not only through providing quality care for the animals of their collection, but also via involvement with SSP and TAG programs (nutrition advisor), through Conservation Grant Fund (CGF) evaluations, through AZA accreditation inspections, and simply through active involvement in the NAG. All of these represent considerations for not only AZA accreditation of the zoo, but also the variety of conservation research metrics requested by AZA annually.

Outreach (consulting, fund- and friend-raising)

A qualified zoo nutritionist is an excellent representative of the zoo and its dedication to quality animal care (simply by their presence, but also given their extensive knowledge of a wide variety of taxa, their detailed knowledge of the zoo's collection, and their experience). Due to the comprehensive nature of a nutritionist's training and background (knowledge of not only nutrition but staff management, statistics, population management, natural history, etc), a qualified zoo nutritionist also can be a well-qualified resource for topics beyond nutrition. Because so few zoos currently employ nutritionists, often they are called upon for assistance from other zoos (a double-edged sword, for several reasons). In this way, they can serve as a direct revenue generator. Indirectly, a trained nutritionist can also generate revenue through participation in marketing or special events. Many institutions promote/sell unique experiences to their members or potential donors. These experiences can range from behind the scene tours of nutrition facilities (most are off public view and consequently of interest for this reason alone), to presentations by staff, to active engagement in activities centered around nutrition and feeding of the collection.

Qualities of a Qualified Zoo Nutritionist

To date, there remains no specific certification or verification process for a qualified zoo nutritionist. The zoo nutritionists who currently are functioning in the field have a wide variety of education credentials, experience levels, and applied backgrounds. There is not one single factor that defines a qualified zoo nutritionist or serves to disqualify someone from the discipline. The long term zoo nutrition programs that exist in North America are directed by individuals with extensive knowledge and experience of all aspects of zoo nutrition (operational, clinical, research, etc), but regardless of directing comprehensive programs or not, they all have common traits leading to their success. Some of the common traits of zoo nutritionists are:

- Demonstrated problem solving ability. We don't know all of the answers, nor will we ever. Being able to apply a wide range of somewhat associated information appropriately to the "issue" at hand is crucial - including the ability to critically review literature (both peer-reviewed and popular press) and extrapolate appropriately.
- Demonstrated basic knowledge of natural history as it relates to nutrition and diet of all taxa included in the animal collection.
- Demonstrated ability to work within a team to problem-solve and think/act decisively
- Advanced degree in and/or definitively/clearly demonstrated knowledge of nutrition (human or animal), and the clear ability to apply information across taxa. Advanced degrees in nutrition or nutrition-related disciplines clearly provide a foundation in problem-solving and research thought processes.
- Demonstrated engagement in the professional community (membership in the NAG and/or CNS – Comparative Nutrition Society, attendance at meetings, etc) to be aware of current topics in the field, for continuing education, to share limited resources, and collaborate when possible.
- Other skills useful to your specific zoo and/or operation (equipment operation, demonstrated inventory management skills, personnel management skills, budget management, laboratory methods/operations, public speaking, publication record, etc).

Ways of Hiring a Qualified Zoo Nutritionist

Once the decision is made to hire a nutritionist for the zoo collection, it is important to access and evaluate as many qualified individuals as possible. Usually, this process begins by clearly defining the institutional goals for the position, developing the position/job description, and defining the desired characteristics (as described in the previous section). Failure to clearly define expectations/responsibilities or a purposeful approach of "we'll see how it develops" (with no guarantees) may not attract qualified individuals. Pay scale should be comparable to positions within the institution with a similar level of responsibility, expectations, education, and experience. To date, five zoo nutritionists have been produced through specific zoo nutrition residency programs, and the SCARF program remains a viable source for a qualified zoo nutritionist (keep in mind that it theoretically produces a graduate/qualified zoo nutritionist every 2-3 years). The AZA Nutrition Advisory Group (NAG) and the Zoo and Wildlife Nutrition Foundation (ZWNF) are excellent points of contact for this program, as well as outlets for position announcements that are targeted for zoo and wildlife nutrition professionals. The Comparative Nutrition Society (CNS), along with the NAG and ZWNF have websites and (in some cases) list serves to more efficiently distribute the announcement. In addition, wildlife-focused online job lists, including AZA, Texas A&M wildlife jobs boards, etc, are excellent ways to distribute announcements to qualified candidates. As a small but growing discipline, consider that you may desire not to target a newly trained nutritionist from the residency program or a recent graduate, but rather a current zoo nutrition professional at another facility around the world. Based on your goals, and the position description complexity, requirements, and/or benefits/compensation, you may be able to hire an experienced zoo nutritionist from an existing program that is looking for a new experience, additional challenges, or a different collection. All of these are viable options. Additionally, attending nutrition conferences such as the NAG/ZWNF biennial conference which runs concurrent with AAZV, can allow an interested institution to meet nutritionists, current and former residents, and students to learn their areas of interest and expertise as well provide an opportunity for communication/interaction.

Resources

The AZA Nutrition Advisory Group is a scientific advisory group established by AZA in 1994 to provide nutrition expertise and guidance to the members of AZA (institutions and individuals). The stated mission was (and remains): to promote the welfare of animals in captivity by incorporating the science of nutrition into their husbandry. It was hoped at the time that, "through the formation of a Nutrition Advisory Group that the role of nutrition in the science of maintaining captive wild animals and sustaining endangered animals in the wild [would] move to the forefront." With the development of a new website format (www.nagonline.net) and the continued efforts of the steering committee and the general membership to serve not only the AZA zoos who employ us, but also the SSP, TAG, and SAG programs within AZA, the Nutrition Advisory Group has continued to grow and provide for the development and diffusion of knowledge to better manage the nutritional care of the animals in our charge. In 2012, the Zoo and Wildlife Nutrition Foundation (ZWNF) was formed. The Foundation was established for the purpose of contributing expertise and providing funds to support the field of zoo and wildlife nutrition worldwide, furthering the science of zoo nutrition, facilitating the dissemination of nutrition knowledge, and developing the next generation of zoo nutrition leaders. The ZWNF functions independently, but supports the goals and mission of the NAG.

The Comparative Nutrition Society (www.CNSweb.org) was founded in 1996 to foster communication among laboratory and field scientists from various disciplines with interests in comparative nutrition. It helps establish and promote a professional concept of comparative nutrition, and encourages education and professional development in the field. All of these groups can serve as resources for those interested in hiring a full time nutritionist – to determine what nutritionists can do, who is available, what skill sets exist and what skill sets are desired, among other useful functions.

A Final Word

Currently around 20 out of the over 200 AZA accredited zoos in North America employ at least one full time nutritionist on staff to focus on the nutritional needs of the animals in those collections. Given the fact that nutrition (provision of a diet) is one of the few daily influences over health and well-being (beyond environment) for the entire life of the animal, employing a qualified and dedicated staff member to focus on those nutrition needs is obvious. As we continue to focus (in word and deed) on animal care and welfare of our collections, it is incumbent upon zoos to hire qualified nutritionists for this critical aspect of appropriate animal care.

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Milliken Meat Products Ltd.

Company Profile Milliken Meat Products Ltd.

Milliken Meat Products Ltd. is a family company producing the Toronto Zoo Carnivore Diets since 1996. First, we produced the diets for the Toronto Zoo and then we expanded our business within Canada and the United States. Today, there are many zoological institutions and conservation programs that use the Toronto Zoo Carnivore Diets; the result of more than 25 years of research and development at the Toronto Zoo.

The Toronto Zoo Carnivore Diets are formulated to be fed as the primary ration in the feeding of carnivores in human care. These diets are horse meat based and are formulated to provide the required nutrients for healthy zoo carnivores.

Manufacturing is in a dedicated and updated facility, in accordance with the current provincial (Ontario) standards of hygiene for meat processing and subjected to periodic oversight by the Toronto Zoo Wildlife Nutrition Centre.

At Milliken Meat Products Ltd we are always ready to satisfy the needs of your "wild" carnivores.

Sincerely,

Jason De Rosa
Milliken Meat Product Ltd, July 14, 2015

Email: millikenmeat@hotmail.com

Phone: (905) 415-0060
Fax: (905) 513 8486

TUCKER'S

RAW FROZEN

ZOOLOGICAL SELECT

Raw Food and Enrichment For Carnivores

Tucker's Raw Frozen & Treats was founded in 2009 based upon the simple premise of quality and custom formulating. Using 100% USDA human grade raw materials we are able to custom blend to each and every customer's and often times specimen's specification.

Tucker's also specializes in sourcing unique forms of enrichment for carnivores. From Bison cages to Elk and Alpaca Tucker's provides the freshest of quality enrichment to all 51 states and Canada.

ANDERSON FEED COMPANY

3338 S Chana Road
Chana, IL 61015
Phone (815) 732-3338
Fax (815) 732-3338
nuzufeed@gmail.com

NuZu feed was founded in 2008 with the idea that we would listen to what was being needed in the zoo nutrition world. We are always taking input from nutritionists in different fields to provide more insight. We, at times, need to take an out of the box approach when we begin to formulate, but as we consider the needs of the species we are working with, what we are trying to obtain in the finished product and the eating habits of the animal, we are able to produce a trial formula.

My background is in feed manufacturing, more specifically quality control. I spent many years working for one of the nation's largest commercial feed companies. During that time, I became friends with several zoos and was asked to do some research formulating. I left my position and started Anderson Feed Company which owns the NuZu trade name.

We now have the following formulas available, Hi Pro @ 15.5% CP, Lo Pro @ 12% CP, Mega Herbivore cubes @11% CP and 4100 IU/lb of active Vit E. for elephants, Low Iron cubes @ 15.5% CP with less than 350 ppm of Iron and a natural chelator for black rhinos, Low Isoflavonoids @15.5% CP for white rhinos, Stabul 1 equine diets @ 12% CP or 14.5% CP have less than 10% starch and sugar and are banana flavored, we also have ratite diets and goat diets. We have just started to carry other companies' products to make it easier for our customers.

We are a fixed formula company and each month we assay the first batch run. Most of our formulas have moderate levels of starch and sugar and higher levels of fiber. We are always willing to look at making a research formulation. Please contact us through our website www.nuzufeed.com for samples or to get more information.

Randy Anderson
Anderson Feed Company
815-732-3338

THE RODENT SOURCE LLC

Email: rodentsource@gmail.com
Phone: (813) 774-0265

The Rodent Source has been in business for over 10 years providing quality feeders to a wide variety of clientel. Such as, zooilogical institutions, pet stores, reptile breeders, non-profit bird rescues, and wholesalers.
Our feeder animals are raised in an open air environment and fed a specialy formulated diet to acheive a level of quality nutrition our clientel has come to expect.

We proudly provide the following…

- Anoles (Brown or green, Frozen)
- Rats (Frozen or Live)
- Mice (Frozen and Live)
- Rabbits (Frozen and Live)
- Chickens (Frozen and Live)
- Quail (Frozen)
- Guinea Pigs (Frozen)

The Zoo and Wildlife Nutrition Foundation

The Zoo and Wildlife Nutrition Foundation (ZWNF) was established in 2012 in support of the AZA Nutrition Advisory Group by a group of individual leaders within the field of zoo and wildlife nutrition. The ZWNF is a non-profit organization established for the purpose of:

- *contributing expertise and providing funds to support zoo and wildlife nutrition programs worldwide,*
- *furthering the science of zoo nutrition,*
- *facilitating the dissemination of nutrition knowledge, and*
- *developing the next generation of zoo nutrition leaders.*

Given the resource-challenged environment within which zoos, aquariums, and wild animal parks must function, the partnership between the AZA NAG and the ZWNF is designed to maximize the impact that professional zoo nutritionists have on the care and management of our current and future collections, as well as on the training and leadership of the next generation of zoo nutritionists.

This collaboration is just in its infancy, but the future appears very bright for the impact both groups can have on the field as a whole, as well as all on the groups (SSPs, TAGs, SAGs, member institutions and AZA as a whole) we collectively serve.

Sponsor Contact Information

Anderson Feed Company / NuZu
3338 S Chana Rd
Chana, IL 61015
815-732-3338
nuzufeed@gmail.com

Australian Outback Plantation
P.O. Box 799
Tonopah, AZ 85354
623-780-8500
australianoutbackplants@gmail.com

Carnivore Essentials
800-890-7039
www.CarnivoreEssentials.com

Central Nebraska Packing, Inc
PO Box 550
North Platte, NE 69103-0550
877-900-3003 / 800-445-2881
www.nebraskabrand.com

Five Points Cricket Farm
4715 Trout Lake Rd
Kempton, PA 19529
484-857-1888
info@fivepointscricketfarm.com

Green Horse, LLC
1818 Millersburg Road
Paris, KY 40361
859-338-4521
www.greenhorsebedding.com

Marion Zoological
763-559-3305
www.marionzoological.com

Mazuri
Land O'Lakes Purina Feed LLC
PO Box 66812
St. Louis, MO 63166
800-227-8941
www.mazuri.com

McRoberts Sales Co, Inc
P.O. Box 489
Ruskin, FL 33575
813-645-2561
www.mcrobertssales.com

Milliken Meat Products Ltd
3447 Kennedy Rd, Unit 1
Scarborough, ON
Canada M1V 3P1
905-415-0060 / 416-299-9600
millikenmeat@hotmail.com

Raw Basics
4074 S. Kinnickinnic Ave.
St. Francis, WI 53235
414-481-9900
www.TuckersBones.com

Reliable Protein Products
21001 N. Tatum Blvd
Ste# 1630-620
Phoenix, AZ 85050
800-253-1330
www.zoofood.com

RodentPro
P.O. Box 118
Inglefield, IN 47618-9998
812-867-7598
www.RodentPro.com

The Rodent Source, LLC
813-774-0265
rodentsource@gmail.com

Smelt Feed and Pet Supply, Inc
4116 E. 7th Ave
Tampa, FL 33605
800-827-2473

Zukudla
12 Old King Rd
Nobleton, ON
Canada L0G 1N0
877-985-8352
www.zukudla.com