Zoo and Wildlife Nutrition Foundation

ZWNF

and

Nutrition Advisory Group

ASSOCIATION OF ZOOS & AQUARIUMS

NAG

Twelfth Conference on Zoo and Wildlife Nutrition

24-27 September 2017
Frisco, Texas, USA

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Proceedings of the

Twelfth 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
Matt Brooks, Amy Coslik, and Ann Ward

24-27 September 2017
Frisco, Texas, USA

2017 Conference Committee

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The Conference Committee wishes to express our sincere thanks to the many people who were instrumental forces behind the twelfth conference on zoo and wildlife nutrition. We also would like to thank AAZV and the other professional organizations present at this meeting for the opportunity to meet simultaneously with them in order to encourage camaraderie and information sharing among the groups.

2017 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, OT Canada
1997	Fort Worth, Texas
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
2017	Frisco, Texas

September 2017

Hello Everyone,

It is with great pleasure that we welcome you to the Zoo and Wildlife Nutrition Foundation and AZA Nutrition Advisory Group's Twelfth Conference on Zoo and Wildlife Nutrition. This time we meet in conjunction with the American Association of Zoo Veterinarians (AAZV), AAFV, ARAV, and AEMV.

With multiple groups meeting together, it is an opportunity to share ideas, further develop collaborations pivotal for the care of our animals, and allow the science of zoo nutrition to be at the forefront of these discussions. We would like to thank Rob Hilsenroth, Adine Nicholson, and the rest of the AAZV team for their assistance and logistical support as we developed the meeting plan. But, remember, regardless of how hard people worked to develop the conference schedule, it is up to YOU to be outgoing and engage your peers for the few days that we share together, in order for these future collaborations to blossom.

This year, we continue the additions from last conference, designed to enhance involvement and participation. Case discussions will continue the increased emphasis on thought process development, problem solving, 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 many dedicated people. The conference committee is listed herein, and the efforts of all of these folks are much appreciated. If you are interested in being involved, the Conference Committee is an excellent way to make that happen. We anticipate discussions about our upcoming conference during our membership meeting.

We received significant financial support from Mazuri, Central Nebraska Packing, Rodent Pro, Coconut Fields Forever, and Milliken Meat Products, among many others. Please make a special effort to thank all of those who supported the conference while you're here – sponsors and exhibitors. They support us with new information and technology that we can use to do our jobs well. The field of zoo nutrition continues to advance and the ongoing importance of science-based nutrition decision making remains paramount.

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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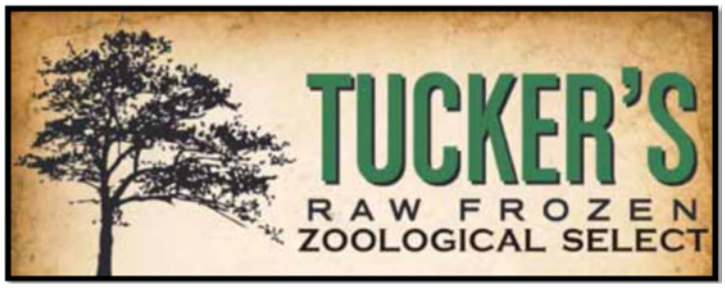

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The Zoo and Wildlife Nutrition Foundation and the Nutrition Advisory Group are appreciative of the support provided from all of our sponsors, big and small. It is through their generous financial support (in many cases, over a period of numerous years, spanning multiple conferences) that we continue to meet and share the science of zoo animal nutrition and nutritional husbandry, as we address the current nutritional issues and better prepare the next generation of zoo nutritionists.

Thank you!

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Crude Protein	%	22	21	22
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Calcium	%	0.44	0.24	0.23
Phosphorous	%	0.43	0.32	0.19
Magnesium	%	0.02	0.02	0.02
Zinc	mg/kg	28	28	29
Taurine	%	0.07	0.06	0.06
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CONTACTS **Mr. Orlando De Rosa and Mr. Jason De Rosa**

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Thank You for the Opportunity to Support Your 12th Biennial AZA/ NAG Workshop. We Look Forward to Hearing From You.

Lynn A. Waidelich, President

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xxx

Sue Crissey Animal Nutrition Residency Fund (SCARF) Benefit

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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 (https://nagonline.net) for details.

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

Nutrition Advisory Group
Twelfth Conference Schedule
23 – 27 September 2017
Embassy Suites Hotel Dallas-Frisco & Conference Center, Frisco, Texas

Saturday, 23 September 2017

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

Sunday, 24 September 2017

8:00 - 9:00 AM	Registration	Reg 2
9 AM – 4 PM	**Workshop: Produce Classifications: Revolutionary considerations for improving zoo animal diet formulation** *INVITED SPEAKER: Erin Vogel* *Presenters: Heidi Bissell, Barbara Henry, Erin Kendrick, Adam Reppert, Barbara Toddes, and Kibby Treiber*	Ranger/Seabiscuit/Traveler
2:00 PM	Poster Set-up	Pre Convene
4:00 - 5:30 PM	Registration	Reg 2
6:00 PM	**Icebreaker with AAZV**	Dallas World Aquarium *Bus departs hotel at 6:00 PM*

Monday, 25 September 2017

6:30 – 7:30 AM	Registration	Pre Convene
8:00 - 8:10 AM	Introductions, welcome, opening with AAZV	Frisco 1 & 2
8:20 – 8:35 AM	NAG Introductions, welcome, opening *Kerri Slifka and Mike Maslanka*	Ranger/Seabiscuit/Traveler
	Herbivore/Browse *Session Chair: Deb Schmidt*	Ranger/Seabiscuit/Traveler
8:35 AM	**Browser Nutrition – Past, present, and future** *INVITED SPEAKER: Jürgen Hummel*, Isabel Gussek, and Marcus Clauss*	

9:15 AM **Analyses of diet and serum mineral concentrations in African elephants (*Loxodonta africana*) housed at the NC zoo**
Jordan Wood, Elizabeth Koutsos, Corinne Kendall, Larry Minter, Alejandra McComb, Troy Tollefson, and Kimberly Ange-van Heugten*
Participant in the Roy McClements Student-Keeper Paper Competition[#]

9:35 AM **Update on the provision of browse at Toronto Zoo**
Jaap Wenswoort, Elizabeth McGregor, Sarra Gourlie, and Benjamin Martin*

9:55 AM **Browse collection and preservation for a winter in a northern zoo**
Jennifer Watts

10:20 -10:50 AM **BREAK and POSTER SESSION** Pre Convene

Life Stage Nutrition Ranger/Seabiscuit/Traveler
Session Chair: Ann Ward

10:50 AM **Proximate composition of milk of captive nine-banded armadillos (*Dasypus novemcinctus*)**
Michael Power, S. Michelle Watts, Katie Murtough, and Frank Knight*

11:10 AM **Handrearing a Nile hippo (*Hippopotamus amphibius*): a rare opportunity for a collaboration**
Barbara Henry, Michael Power, and Michael Maslanka*

11:30 AM **Effect of different protein levels on the performance and apparent protein digestibility of orphan calves of Amazonian manatees (*Trichechus inunguis*)**
Pierina Mendoza, Rony Riveros, and Carlos Vilchez*

11:50 AM **Evaluation of diet influence on oxidative stress and its impact on semen quality in snow leopards (*Uncia uncia*)**
Cayla Iska, Cheryl Morris, and Jason Herrick*

12:10 – 1:35 PM **LUNCH ON YOUR OWN**

Primates Ranger/Seabiscuit/Traveler
Session Chair: Adam Reppert

1:35 PM **Baseline intake study for four species of primates in captivity: *Callithrix pygmaea*, *Nycticebus pygmaeus*, *Propithecus coquereli*, and *Callithrix geoffroyi***
Timothy Brunner and Barbara Toddes*
Participant in the Roy McClements Student-Keeper Paper Competition[#]

1:55 PM **Vitamin D status of wild toque macaques in Sri Lanka**
Michael Power and Wolfgang Dittus*

2:15 PM **Case Discussion - Implications of managing a mother-reared orangutan indoors during early infancy**
Erin Kendrick and Michael Maslanka*

2:35 PM **Case Discussion - Regurgitation and reingestion in captive great apes**
Kelly Kappen, Cayla Iska, Roni McClellen, and Cheryl Morris*

2:55 - 3:25 PM **BREAK and POSTER SESSION** Pre Convene

Aquatics Ranger/Seabiscuit/Traveler
Session Chair: Ann Ward

3:25 PM **Tales from an aquatic nutritionist: Applying field studies on elasmobranchs to managed collections**
INVITED SPEAKER: Lisa Hoopes

4:05 PM **Blood fatty acid profiles of juvenile wild green turtles (*Chelonia mydas*) and Kemp's ridley turtles (*Lepidochelys kempii*)**
Elizabeth Koutsos, Jb Minter, Kimberly Ange-van Heugten, Johanna Mejia-Fava, and Craig Harms*

4:25 PM **Comparative serum analysis of free-ranging and managed green moray eels (*Gymnothorax funebris*) and relationship to diet fed to eels under human care**
Amanda Ardente, Scott Williams, Natalie Mylniczenko, John Dickson, Alisha Fredrickson, Christy Macdonald, Forrest Young, Kathleen Sullivan, Shannon Livingston, James Colee, and Eduardo Valdes*

4:45 PM **Impact of removing dietary supplementation on serum nutrient concentrations in a managed population of southern stingray (*Dasyatis americana*)**
Scott Williams, Amanda Ardente, Natalie Mylniczenko, Tristan Guttridge, Kathleen Sullivan, Shannon Livingston, and Eduardo Valdes*

5:05 PM **DINNER ON YOUR OWN**

7:00 – 9:00 PM **SCARF BENEFIT with "Trivia & Treats"** Ranger/Seabiscuit/Traveler

Tuesday, 26 September 2017

Disease Management Ranger/Seabiscuit/Traveler
Session Chair: Ann Ward

7:55 AM **Clinical significance of nutritional issues**
INVITED SPEAKER: Donald Neiffer

9:15 AM **Rhabdomyolysis in captive pelicans: conflicting indicators of vitamin E status**
Kibby Treiber and Ann Ward

9:35 - 10:00 AM **BREAK and POSTER SESSION** Pre Convene

10:00 AM **Gastritis in the short-beaked echidna (*Tachyglossus aculeatus*): Parallels with ruminal acidosis in herbivores and implications for the understanding of echidna digestive physiology**
Lydia Tong, Michelle Shaw, Deborah Chong, Gabrielle Tobias, Frances Hulst, Kimberly Herrin, and Larry Vogelnest*

10:20 AM **Short-beaked echidnas (*Tachyglossus aculeatus*): An insectivorous herbivore**
Michelle Shaw, Lydia Tong, Phoebe Meagher, Kate Brandis, and Debashish Mazumder*

10:40 AM **Case Study: Winos for rhinos: feeding grape pomace to black rhinoceros (*Diceros bicornis*) as a method for mitigating iron storage disease**
Matthew Brooks, Bob Lee, and Jeffrey Pera*

11:00 AM **Centralization of knowledge for disease understanding and prevention: Center of excellence for iron overload disorder in browsing rhinos pilot toolkit**
Kathleen Sullivan, Shana Lavin, Mandi Schook, Katie Leighty, Shannon Livingston, Mark Kamhout, Geoff Pye, Tammie Bettinger, and Eduardo Valdes*

11:20 AM **Case Discussion – The Mob – Under investigation**
Amanda Ardente, Ryan De Voe, Deidre Fontenot, Joy Gonzalez, Kathleen Sullivan, Shannon Livingston, Scott Williams, and Eduardo Valdes*

11:40 AM **Program updates**
Coordinator: Mike Maslanka

12:00 PM **ZOO DAY** Dallas Zoo
Bus departs hotel at 12:15 PM

Wednesday, 27 September 2017

Assessment & Laboratory Operations
Session Chair: Liz Koutsos

7:55 AM **Establishing evolutionarily derived levels of vitamin D through metabolic profiles in primates**
INVITED SPEAKER: Toni Ziegler

8:35 AM **Sequencing the black rhino L-ferritin gene: How accurate is our testing?**
Kathleen Sullivan, Richard Coffey, Shana Lavin, Shannon Livingston, Lori Warren, Eduardo Valdes, and Mitchell Knutson*

8:55 AM **Continuing assessment of vitamin analysis reliability across laboratories: Examples in white and black rhino species**
Kathleen Sullivan, Amanda Ardente, Scott Williams, Shannon Livingston, and Eduardo Valdes*

9:15 AM **Program updates**
Coordinator: Mike Maslanka

9:35 - 10:20 AM **BREAK and VISIT EXHIBITORS** Pre Convene/Frisco 6

Insectivore/Carnivores Ranger/Seabiscuit/Traveler
Session Chair: Matt Brooks

10:20 AM **Macronutrient selection in mammalian insectivores at Busch Gardens Tampa Bay**
Heidi Bissell

10:40 AM **Development and application of a new insect-based complete diet for insectivorous mammals**
Jennifer Watts

11:00 AM **Carotenoid gut-loading of crickets (*Acheta domestica*) and mealworms (*Tenebrio molitor*)**
Erin Kendrick and Michael Maslanka*

11:20 AM **Do pinhead crickets have the amino acid profile to support growth in La Hotte bush frogs (*Eleutherodactylus bakeri*)?**
Barbara Toddes

11:40 AM **Grizzly bear (*Ursus arctos*) diet management: Seasonal diets to attain healthy weight**
Katherine Kerr, Hali O'Connor, Jacob Shanks, Gaylene Thomas, and Chris Hamlin*

12:00 PM **From farm to fang: Sourcing carcasses for carnivores**
Katherine Kerr, Jessica Sheftel, Meg Sutherland-Smith, Meredith Clancy, Lauren Howard, and Andrea Fidgett*

12:20 - 2:00 PM **LUNCH ON YOUR OWN**

Commissary Operations Ranger/Seabiscuit/Traveler
Session Chair: Kerri Slifka

2:00 PM **Program updates**
Coordinator: Mike Maslanka

2:20 PM **Doing more with less at Busch Gardens Tampa Bay: Improving commissary efficiency**
*Clifton Martel and Heidi Bissell**

2:40 PM **An examination of various methods of diet packaging**
Kerri Slifka

3:00 PM **Bringing a new level of green to the nutrition center - The road to zero waste certification at Disney's Animal Kingdom**
Shannon Livingston, Debbie Weber, Elisabeth Brunk, and Eduardo Valdes*

3:20 – 3:45 PM **BREAK and VISIT EXHIBITORS** Pre Convene/Frisco 6

3:45 – 4:45 PM **NAG MEMBERSHIP MEETING** Ranger/Seabiscuit/Traveler
Mike Maslanka

6:30 PM **NAG BANQUET** Ranger/Seabiscuit/Traveler

[#]The Roy McClements Student-Keeper Paper Competition is sponsored by
Central Nebraska Packing, Inc.

Visit with the authors Monday 9/25 at 10:20 AM and 2:55 PM and Tuesday 9/26 at 9:35 AM.

Practical investigation of cricket dust supplements commonly used to enhance diets provided to insectivore species under human care
Amanda Ardente, Kathleen Sullivan, Shannon Livingston, Scott Williams, James Colee, and Eduardo Valdes*

Development of an artificial diet to support conservation efforts of the Atala hairstreak butterfly (*Eummaeus atala*)
Amanda Ardente, Jamie Sincage, Zak Gezon, Ann Savage, and Eduardo Valdes*

The influence of giraffe behavior on parasite load: Impact of husbandry modifications at Busch Gardens Tampa Bay
Nancy Arnold, Connor Cain, Robbin Rowland, Samantha Steele, Cara Martel, Michael Burton, and Heidi Bissell*

Analysis of fecal glucocorticoid concentration in African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants in relation to management and nutritional factors
Jessica Bray, Kimberly Ange-van Heugten, David Dickey, Charlotte Farin, Kathy Carlstead, and Janine Brown*

Gut-loading diet evaluation for crickets (*Acheta domesticus*), mealworms (*Tenebrio molitor*), and superworms (*Zophobas morio*) for the purposes of optimizing institutional protocols
Matthew Brooks and Gwen Harris*

Analysis of fatty acid profiles in eastern box (*Terrapene carolina carolina*) and common snapping (*Chelydra serpentine*) turtles for wild and in-human care environments
Khushboo Dass, Kimberly Ange-van Heugten, Elizabeth Koutsos*, and Larry Minter*

Using straw notes to standardize and improve zoo nutrition outcomes
Andrea Fidgett, Katherine Kerr, and Michele Gaffney*

A simple, practical method for measurement of fat in milk, applied to samples from mid- to late-lactating working elephants in a maternity camp in Myanmar
Yadana Aung Myo Han, Ellen Dierenfeld, Khyne Mar, Mirkka Lahdenperä, Virpi Lummaa, and Aung Aung*

Case study: Iron in black rhinoceros diets: The impact of pasture
Jasmyn Mimiko, Elizabeth Stringer, and Jennifer Parsons*
Participant in the Roy McClements Student-Keeper Paper Competition[#]

Macronutrient composition of milk from an Asian rhinoceros
Katie Murtough, Michael Power, and Ann Ward*

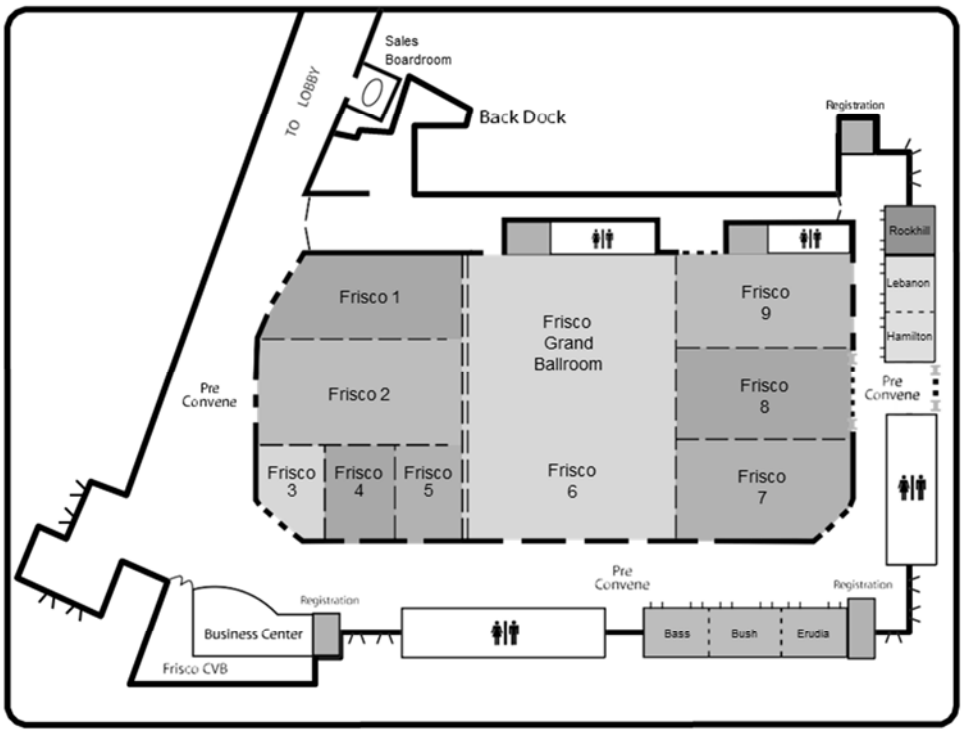
Estimating apparent dry matter digestibility using dietary manganese in the common marmoset (*Callithrix jacchus*)
Michael Power, Caitlin Arlotta, Jessica Davis, Ricki Colman, and Suzette Tardif*

Edible centerpieces: Reduce, Reuse, Recycle
Debra Schmidt and Barbara Henry**

Technology use for physiological state: Use of NIRS to predict pregnancy status in bongo (*Tragelaphus eurycerus*)
Kathleen Sullivan, Shana Lavin, Kelsey Hall, Avnee Mistry*, Scott Williams, Shannon Livingston, Catharine Wheaton, and Eduardo Valdes*

#The Roy McClements Student-Keeper Paper Competition is sponsored by **Central Nebraska Packing, Inc**.

EMBASSY SUITES FRISCO – CONFERENCE CENTER MAP



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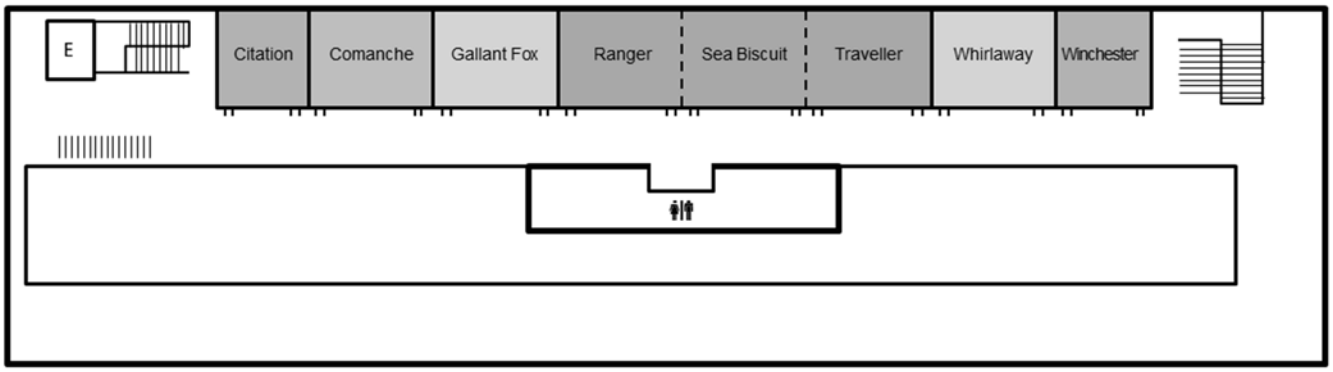

CONFERENCE CENTER 2nd FLOOR

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In order of first author's last name

THE MOB – UNDER INVESTIGATION

Amanda Ardente, DVM, PhD[1,2]*, Ryan De Voe, DVM, Dipl. ACZM[1], Deidre Fontenot, DVM[1], Joy Gonzalez[3], Kathleen Sullivan, PhD[1], Shannon Livingston, MSc[1], Scott Williams, MS[1], Eduardo Valdes, PhD[1,2,4,5]

[1]Department of Animal Health, Disney's Animals, Science, and Environment, 1180 N. Savannah Circle, Bay Lake FL 32830, USA
[2]Department of Animal Sciences, PO Box 110910, University of Florida, Gainesville, FL 32608, USA
[3]Disney's Animals, Science, and Environment, 1180 N. Savannah Circle, Bay Lake FL 32830, USA
[4]University of Guelph, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada
[5]University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816, USA

'Brewer', a 4 year old male red kangaroo (*Macropus rufus*) under human care at Disney's Animal Kingdom, presented on December 10, 2016 with signs of colic, penile prolapse, and dribbling urine. Diagnostic imaging, including radiographs and computed tomography (CT) with contrast, revealed urethral obstruction with a radiopaque stone and additional uroliths in the left ureter and right renal pelvis. The urethral and ureteral uroliths were surgically removed, and supportive care was provided, including intravenous fluids, antibiotics, and anti-inflammatories. Unfortunately the stone in his kidney could not be removed. 'Brewer' recovered well, but on March 31, 2017, he presented again with similar clinical signs indicating acute urethral obstruction. Medical and surgical management was initiated, as previously described. 'Brewer' is one of eight red kangaroos cared for at Disney's Animal Kingdom, so following his second urinary obstruction, presence of urolithiasis was investigated for the entire kangaroo mob. Various factors influence whether uroliths develop in the urinary tract of mammals, but diet can be a major contributor. Understanding which questions are important to ask and how to best obtain the information is key to discerning whether this is an individual clinical case or a herd-health issue.

Points to Discuss:

- How would one approach this case as a potential herd-health issue?
- What husbandry questions would be important to ask?
- What dietary questions would be important to ask, and what samples would be needed to answer those questions?
- What non-diet related questions would be important to ask, considering urolithiasis in other species?
- How could you modify management of the herd to mitigate the clinical disease process?

DEVELOPMENT OF AN ARTIFICIAL DIET TO SUPPORT CONSERVATION EFFORTS OF THE ATALA HAIRSTREAK BUTTERFLY (*EUMAEUS ATALA*)

Amanda Ardente, DVM, PhD[1,2]*, Jamie Sincage[3], Zak Gezon, PhD[4], Ann Savage, PhD[4], Eduardo Valdes, PhD[1,2,5,6]

[1]Department of Animal Health, Disney's Animals, Science, and Environment, 1180 N. Savannah Circle, Bay Lake FL 32830, USA
[2]Department of Animal Sciences, PO Box 110910, University of Florida, Gainesville, FL 32611, USA
[3]Disney's Animals, Science, and Environment, 1180 N. Savannah Circle, Bay Lake FL 32830, USA
[4]Department of Conservation Programs, Disney's Animal Kingdom, 1180 N. Savannah Circle, Bay Lake FL 32830, USA
[5]University of Guelph, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada
[6]University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816, USA

Abstract

The rare Atala hairstreak butterfly (*Eumaeus atala*) is native to Florida, and coontie (*Zamia floridana*), a small cycad, is its only native host plant. In the early 1900's, coontie was eradicated due to unsustainable agricultural practices, so Atala populations plummeted. The butterfly was thought to be extinct in the mid-1900s but has seen a comeback since its rediscovery near Miami in the 1970s. As part of a conservation effort, Disney's Animal Kingdom is raising Atala butterflies for release into the wild. A challenge with raising the South Florida insects in Central Florida, however, is a shortened coontie growing season. Caterpillars consume only the plants' new growth, which is not abundant during winter months. Thus, a year-round food source would facilitate insect production and increase the impact of the conservation program. We therefore developed an artificial diet to raise Atala caterpillars. Diet trials utilized commercial caterpillar diets with freeze-dried, ground coontie incorporated at different concentrations. Artificial diet success was assessed by comparing caterpillar growth, frass production, time to pupation, pupal weights, emergence, and egg-laying with control caterpillar populations raised on coontie. An artificial diet containing 50% commercial hornworm diet and 50% freeze-dried coontie has shown the most promise for future use. Caterpillar growth, pupal weights, emergence, and egg-laying were comparable to control caterpillars. Rate of caterpillar growth and time to pupation, however, were notably delayed by approximately one week in the 50% coontie diet group when compared to control caterpillars. Frass production was also much lower in artificial diets, possibly due to higher diet digestibility. Lower concentration coontie diets resulted in extremely delayed development and low survivorship. Future trials are being conducted to assess whether completion of a full life cycle is possible on an artificial diet and determine the best method for large scale Atala production using an artificial diet.

Acknowledgements

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Dr. Kathleen Sullivan, Shannon Livingston, and Scott Williams for their support of the project. Lastly, we are grateful for the help of Dr. Lori Warren, Equine Nutritionist from the University of Florida Animal Sciences department in Gainesville, FL, for permitting us the use of her lab freeze-dryer and for her generous assistance in processing the ground coontie samples.

PRACTICAL INVESTIGATION OF CRICKET DUST SUPPLEMENTS COMMONLY USED TO ENHANCE DIETS PROVIDED TO INSECTIVORE SPECIES UNDER HUMAN CARE

Amanda Ardente, DVM, PhD[1,2]*, Kathleen Sullivan, PhD[1], Shannon Livingston, MSc[1], Scott Williams, MS[1], James Colee[3], Eduardo Valdes, PhD[1,2,4,5]

[1]Department of Animal Health, Disney's Animals, Science, and Environment, 1180 N. Savannah Circle, Bay Lake FL 32830, USA
[2]Department of Animal Sciences, PO Box 110910, University of Florida, Gainesville, FL 32611, USA
[3]Statistics Department, McCarty Hall, University of Florida, Gainesville, FL 32611, USA
[4]University of Guelph, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada
[5]University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816, USA

Abstract

Amphibians and reptiles commonly managed under human care are commonly fed farmed feeder crickets (*Acheta domesticus*) that are deficient in calcium. Calcium deficiency can lead to the development of nutritional metabolic bone disease in animals consuming the crickets; therefore, feeder crickets are commonly supplemented with calcium by either dusting the crickets' exoskeleton or by providing crickets with a calcium enriched diet. Experiments evaluating the efficacy of dusting provide variable results, so, we sought to evaluate the nutrient content of feeder crickets commonly fed to insectivores at Disney's Animal Kingdom after dust supplementation. Our objective was to determine which of three dust supplements adhered the best to crickets and optimized the cricket nutrient content at various time points, while mimicking the practical dusting method used at Disney's Animal Kingdom. Three dust supplements, including the product prepared by Disney's Animal Nutrition Center and two commercially available dusting supplements (Repashy and Rep-Cal), were tested for adherence at 4 time points (0, 30, 90, 180 minutes). Nutrient composition of the dusted crickets was then compared across time points and to un-dusted crickets. Based on assessment of cricket weight, all dust treatments had the greatest adherence when measured at time point 0 min. As crickets spent more time in the shaker, dust adherence decreased. All dusted crickets had significantly greater concentrations of Ca ($P \leq 0.05$) when compared to un-dusted control crickets, and Ca concentrations decreased over time for all dusted crickets. Compared to the other two supplements, Repashy achieved the greatest Ca concentration and vitamin A concentration, as it was the only supplement tested containing the vitamin. Repashy also appeared grossly stickier that the Disney and Rep-Cal products. This study assessed the importance of measuring both adherence of dust supplements and resulting nutrient content of feeder crickets over time, using practical application methods, in order to select a product that will achieve the desired cricket nutrient content for insectivore diets.

Introduction

Amphibians and reptiles are commonly managed under human care, either privately owned or as part of a larger collection maintained at a zoo, aquarium, educational facility, or conservation program. It is well-described that farmed feeder crickets (*Acheta domesticus*) commonly fed to these species under human care are deficient in calcium, particularly in relation to a relatively greater phosphorous content (Allen and Oftedal, 1989; Finke, 2003; Oonincx and Dierenfeld,

2012). This inverse ratio of calcium to phosphorous (Ca:P) leads to nutritional metabolic bone disease, which results in stunted growth, osteomalacia, skeletal fractures, neurologic symptoms, and death (Modzelewski *et al*., 1974; Dierenfeld *et al*., 1995; Ferguson *et al*., 1996; Miller *et al*., 2001; Mader, 2006; Hoby *et al*., 2010).

In order to prevent nutritional metabolic bone disease in amphibians and reptiles under human care, the diet is commonly supplemented with calcium to increase the Ca:P to 1:1 - 2:1 (Allen and Oftedal, 1989). Supplementation practices, however, present an ongoing challenge (Trusk and Crissey, 1987; Allen and Oftedal, 1989; Finke, 2003; Sabatini *et al*., 1998; Coslik *et al*., 2009; Sullivan *et al*., 2009). There are two common methods by which insects are supplemented with calcium. The first entails feeding insects a nutrient-rich diet (termed gut-loading), and the second involves applying a powder (or dust) that adheres to the outside of the insect. Both forms of supplementation are administered to insects immediately prior to delivery as prey. Gut-loading has been well-studied and has proven successful in its ability to increase the calcium content of insects (Allen and Oftedal, 1989; Sabatini *et al*., 1998; Finke, 2003; Schlegel *et al*., 2005; Coslik *et al*., 2009). Experiments evaluating the efficacy of dusting, however, are sparser, and results are variable (Sabatini *et al*., 1998; Li *et al*., 2009; Sullivan *et al*., 2009; Michaels *et al*., 2014). Live crickets are reported to clean over 50% of the supplement off of their bodies within 2.5 minutes of being dusted and may only retain 10% of the dust before being consumed by the animal (Li *et al*., 2009; Sabatini *et al*., 1998). The most recent study was conducted in the United Kingdom and used three dusting supplements: Vetark Professional Nutrobal, Exo Terra Calcium + D3, and Zoo Med Repti Calcium with D3 (Michaels *et al*., 2014). No difference was found in the Ca:P ratio of dusted black field crickets (*Gryllus bilmaculatus*) and silent crickets (*G. assimilis*) among the three supplements used, and a Ca:P of greater than 1:1 in the crickets was achieved up to 5.5 hours following dust application. Michaels *et al.* (2014) concluded that dusting, irrespective of supplement brand, was a successful method to provide calcium supplementation to feeder crickets fed to amphibians and reptiles, but that time, species, and instar stage impacted cricket calcium content.

We sought to evaluate the nutrient content of the species and life stage of feeder crickets commonly fed to amphibians and reptiles at Disney's Animal Kingdom after supplementation with the dust prepared by the Animal Nutrition Center and two common dust supplements commercially available in the United States. Our objective was to determine which of the three dust supplements adhered the best to crickets and optimized the cricket nutrient content. We hypothesized that initial adherence would vary among supplements but adherence of all three treatments would decrease over time. We also hypothesized that each of the three supplements would achieve a different nutrient profile in the feeder crickets based on their varied nutrient compositions.

Materials and Methods

Two of the dusting supplements tested for adherence and ability to improve the nutrient profile of feeder crickets were commercially available: Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056) and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031). The third supplement tested was a proprietary reptile blend formulated by the nutritionist at the Disney's Animal Nutrition Center (Disney's Animal Kingdom, Lake Buena Vista, FL 32830). All dusting supplements were analyzed for gross energy, proximate, and mineral analysis (Dairy One Forage Laboratory, 730 Warren Rd., Ithaca, NY 14850 USA),

and vitamins A and E (Covance Inc., Princeton, NJ 08540). Separate groups (Rep 1, $n = 6$; Rep 2, $n = 12$) of adult feeder crickets (¾" size, The Gourmet Rodent, Newberry, FL 32669) were weighed and placed into Cricket Shakers (Rep-Cal Research Labs) with each of the three dust treatments. To replicate standard operating procedures of the Disney's Animal Kingdom herptile team, Cricket Shakers were used to dust crickets, and the experiment was conducted at ambient temperature (75.7-81.1^{0}F) and humidity (48-53%).

Twelve Cricket Shakers, each with 12 g of crickets per shaker, were used for each dusting treatment. The starting weight of the dust in the shaker holding cup was recorded. Dust was applied by inverting the shaker 10 times until crickets were well-coated. Crickets were retained in the shaker for four distinct time periods: 0 min (immediate), 30 min, 90 min, and 180 min. The designated time periods were selected based on observations by the Disney's Animal Kingdom herptile team and reflected the number of minutes that may elapse between dust application and consumption of the insect by an amphibian or reptile. When the given time period elapsed, dusted crickets were transferred into a tared quart size ziplock freezer bag to obtain the final weight on a gram scale. The dust remaining in the cup was also weighed and recorded. For each treatment, crickets from the 12 shakers were pooled into one Ziplock back that was immediately placed into a -20°C freezer. Frozen crickets were shipped overnight with icepacks for gross energy, proximate, and mineral analyses (Dairy One), and for vitamin A and E analyses (Covance). This experiment was done over 4 weekdays within a 2-week period in order to account for any environmental variability and to accommodate for limitations with supply ordering and manpower.

The differences in nutrient concentration between control crickets and treated crickets were calculated, and those differences were compared both within treatment and within time-point (PROC GLIMMIX, SAS University© 2012-2016). Nutrient composition of dust supplements were described based on one analysis performed. Due to variability not accounted for in the initial power analysis performed, significant differences in dust adherence and the effect of time could not be determined, so differences were also described.

Results

The nutrient analysis of dusting supplements varied in crude protein, fat, calcium, phosphorous, and vitamins A and E concentration (Table 1). Disney's proprietary blend dust supplement had the greatest crude protein and fat content but lowest calcium concentration compared to the other two supplements. On the other hand, Rep-Cal had the lowest crude protein and fat contents but the greatest calcium concentration, with negligible phosphorous and vitamin E concentrations and no vitamin A. The crude protein, fat, and calcium contents of Repashy fell between that of Rep-Cal and Disney's proprietary blend, but Repashy had the greatest vitamin A and E concentrations compared to the other two supplements.

Dust adherence measurements were highly variable. Based on assessment of cricket weight, all treatments had the greatest adherence when measured at time point 0 min (Figure 1). As crickets spent more time in the shaker, dust adherence decreased, but assessment in latter time-points was less reliable based on cricket weight measurement alone. Alternatively, the weight of the dust remaining in the cup appeared to be a more reliable measure (Figure 2). Based on gross observation, the Repashy supplement was stickier than the other two products. Repashy also had the greatest adherence compared to Rep-Cal and Disney's proprietary blend dust supplements at

every time point, with the exception of time 0. For this time point, Rep-Cal had slightly greater adherence than the other two dust supplements, but Rep-Cal also was the most variable in its adherence as measured over time.

Nutrient concentration differences between dusted crickets and control (un-supplemented) crickets varied based on supplement (Table 2) and time. All dusted crickets had greater ($P \leq 0.05$) gross energy, neutral detergent fiber (NDF) and calcium (Ca) than control crickets (Table 2). Crickets dusted with Disney's proprietary blend and Repashy supplements had similar vitamin E concentrations that were greater ($P \leq 0.05$) than control crickets. Repashy also had greater ($P \leq 0.05$) dry matter (DM) and vitamin A concentrations than control crickets. Time-point zero and time-point 180 min. had greater ($P \leq 0.05$) DM than control crickets, and time-point zero also had greater ($P \leq 0.05$) crude protein (CP) concentrations compared to control crickets. The only supplement that had a greater ($P \leq 0.05$) concentration of vitamin A than control crickets was Repashy at all time-points (Repashy, μ=3.5 ± 1.5 IU/g vit A As-Fed vs Control, not detectable). Concentrations of Ca and vitamin A decreased over time, as expected, for all dusted crickets (Table 3).

Discussion

One way to improve the calcium content of feeder crickets fed to amphibians and reptiles is to supplement crickets with application of a topical dust containing calcium. The efficiency of nutrient intake by the animals consuming the dusted crickets is variable and relies largely on the inherent stickiness of and nutrient concentrations within the dust product, environmental temperature and humidity, and the time between dust application and consumption by the animal. We analyzed the adherence of three dust supplements and resulting cricket nutrient content at several time-points in order to replicate the practical minimum and maximum amount of time it may take from dust application to consumption.

Crickets dusted at time-point zero had the greatest amount of dust adhered and greatest nutrient content over crickets dusted at all other time-points. Cricket weights, however, were an unreliable measure of dust adherence with greater time lapsed, possibly because of cannibalism and loss of body parts through the Cricket Shaker sieve. Weighing the dust remaining in the cup after shaking and time lapsed was a better measure of adherence. We saw the greatest loss of dust from the system at time-point zero, which means the most dust was adhered to the crickets at that time-point. The loss of dust then decreased with increasing time-points. The 180 minute time-point, however, had more variable results, potentially due to increased cannibalism and loss of body parts that fell through the sieve into the cup holding the remaining dust.

Nutrient concentration differences among dusted crickets and control crickets, however, irrespective of time-point, validate the benefit of dust supplementation, even if 3 hours has lapsed between dust application and consumption. All dusted crickets had significantly greater concentrations of Ca ($P \leq 0.05$) when compared to un-dusted control crickets. Repashy appeared to be grossly stickier and achieved the greatest Ca and vitamin A contents compared to the two products tested.

Although nutrient requirements of reptiles and amphibians have yet to be well-described, we believe it is important to ensure feeder crickets are supplemented with Ca and vitamin A to help

prevent occurrence of the more commonly described reptile diseases. This study assessed the importance of measuring both adherence of dust supplements and resulting nutrient content of feeder crickets over time, using practical application methods, in order to select a product that will achieve the desired cricket nutrient content for insectivore diets. Further studies may be warranted as other products emerge on the commercial market to determine which dust supplement may be preferred based on adherence and nutrient content.

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Table 1. Nutrient analysis of three dust supplements: Disney's Animal Kingdom proprietary blend and two commercially available products.

Dust Supplement[1]	GE[2] kcal/100g As-Fed	DM[2] % As-Fed	CP[2]	NDF[2]	CF[2]	Ca[2]	P[2]	Ca:P	Vitamin A IU/g As-Fed	Vitamin E mg/kg As-Fed
			% Dry-Matter							
Proprietary blend	1.7	91.1	14.0	4.9	1.9	27.0	0.23	117	69	11900
Repashy	1.4	97.1	7.8	8.9	1.7	28.5	0.36	79	313	3930
Rep-Cal	1	99.4	3.8	15.1	0.3	39.5	0.03	1317	ND[2]	6

[1]Proprietary blend (Disney's Animal Kingdom, Lake Buena Vista, FL 32830); Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056); and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031)
[2]GE, gross energy; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; CF, crude fat; Ca, calcium; P, phosphorous; ND, not detectable

Table 2. Gross energy and nutrient concentrations of crickets dusted at initial time-point zero and un-dusted control crickets.

Dust Supplement[1]	GE[2] kcal/100g As-Fed	DM[2] % As-Fed	CP[2]	NDF[2]	CF[2]	Ca[2]	P[2]	Ca:P	Vitamin A IU/g As-Fed	Vitamin E mg/kg As-Fed
			% Dry-Matter							
Proprietary blend	5.6^{b}	25.9^{ab}	71.7	19.7^{b}	16.2	1.4^{a}	0.94	1.5	ND[2]	188^{a}
Repashy	5.1^{b}	26.0^{a}	64.8	19.7^{b}	14.9	5.1^{a}	0.90	5.7	5.6	115^{a}
Rep-Cal	5.1^{b}	25.1^{ab}	66.7	18.9^{b}	14.9	4.3^{a}	0.88	4.9	ND[2]	21^{b}
Control	5.9^{a}	22.6^{b}	76.2	24.5^{a}	16.2	0.1^{b}	1.04	0.1	ND[2]	18^{b}

[1]Proprietary blend (Disney's Animal Kingdom, Lake Buena Vista, FL 32830); Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056); and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031)
[2]GE, gross energy; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; CF, crude fat; Ca, calcium; P, phosphorous; ND, not detectable
[ab]Values with similar superscripts within the same column are similar ($P \leq 0.05$)

Table 3. Gross energy and nutrient concentrations of dusted crickets analyzed at each of four time-points compared with un-dusted control crickets at initial time-point zero.

Dust Supplement[1]	Time (min)	GE[2] kcal/100g As-Fed	DM[2] % As-Fed	CP[2]	NDF[2]	CF[2]	Ca[2]	P[2]	Ca:P	Vitamin A IU/g As-Fed	Vitamin E mg/kg As-Fed
						% Dry-Matter					
Proprietary blend	0	5.6	25.9	71.7	19.7	16.2	1.4	0.94	1.5	ND[1]	188
Proprietary blend	30	5.6	23.6	73.6	23.6	14.1	1.5	0.97	1.5	ND	212
Proprietary blend	90	5.7	23.5	72.9	18.4	14.5	1.4	1.01	1.4	ND	245
Proprietary blend	180	5.6	25.1	73.2	26.3	14.9	1.5	0.98	1.5	ND	280
Repashy	0	5.9	26.0	64.8	19.7	14.9	5.1	0.90	5.7	5.6	115
Repashy	30	5.9	24.4	70.4	21.0	12.7	3.8	0.96	4.0	3.1	70
Repashy	90	5.1	25.0	69.4	24.3	15.3	3.1	0.93	3.3	2.3	73
Repashy	180	5.2	25.7	68.3	25.2	13.8	3.8	0.92	4.1	3.0	79
Rep-Cal	0	5.5	25.1	66.7	18.9	14.9	4.3	0.88	4.9	ND	21
Rep-Cal	30	5.3	23.4	74.1	20.9	14.5	1.5	0.98	1.5	ND	23
Rep-Cal	90	5.1	23.0	72.7	19.6	15.9	2.1	0.98	2.1	ND	24
Rep-Cal	180	5.6	24.6	70.9	22.0	12.7	3.3	0.92	3.6	ND	14
Control	0	5.9	22.6	76.2	24.5	16.2	0.1	1.04	0.1	ND	18

[1]Proprietary blend (Disney's Animal Kingdom, Lake Buena Vista, FL 32830); Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056); and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031)

[2]GE, gross energy; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; CF, crude fat; Ca, calcium; P, phosphorous; ND, not detectable

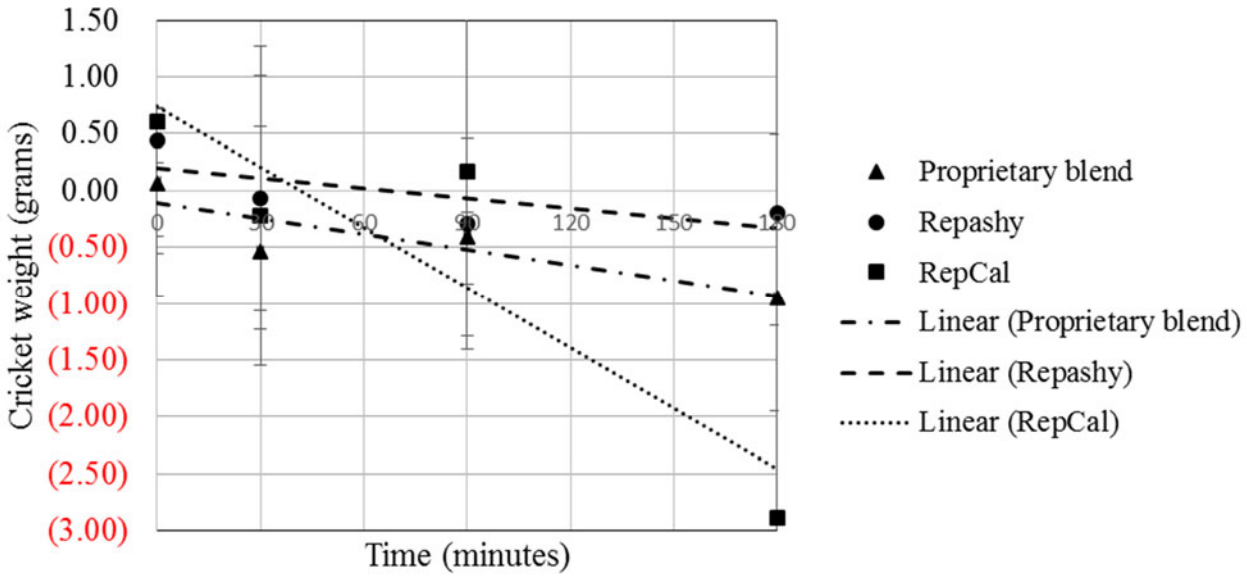

Figure 1. Weight measurements (g) of crickets dusted with supplements, proprietary blend (Disney's Animal Kingdom, Lake Buena Vista, FL 32830), Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056), and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031), at time-points 0, 30, 90, and 180 minutes. All treatments had the greatest adherence when measured at time point 0 min, and as crickets spent more time in the shaker, dust adherence decreased. Nevertheless, assessment at the latter time-points was less reliable based on cricket weight measurement alone.

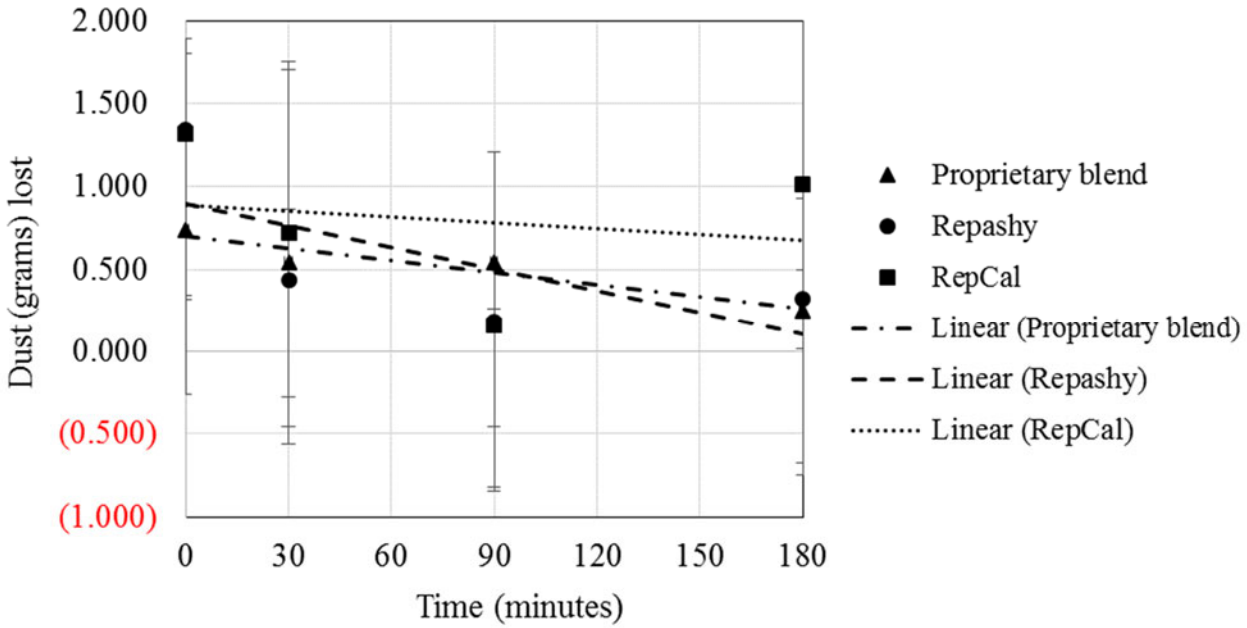

Figure 2. Loss of dust (g) from the Cricket Shaker cup for each of the three dust supplements, proprietary blend (Disney's Animal Kingdom, Lake Buena Vista, FL 32830), Repashy Calcium Plus LoD (Repashy Ventures, Inc., Oceanside, CA 92056), and Rep-Cal Calcium, Phosphorous-Free, No Vitamin D_3 (Rep-Cal Research Labs, Los Gatos, CA 95031), at time-points 0, 30, 90, and 180 minutes. The greatest loss of dust (g) from the system was observed at time-point zero, which means that the most dust was adhered to the crickets at that time-point. The loss of dust then decreased with increasing time-points. The 180 minute time-point, however, had more variable results, potentially due to increased cannibalism and loss of body parts that fell through the sieve into the cup holding the remaining dust.

COMPARATIVE SERUM ANALYSIS OF FREE-RANGING AND MANAGED GREEN MORAY EELS (*GYMNOTHORAX FUNEBRIS*) AND RELATIONSHIP TO DIET FED TO EELS UNDER HUMAN CARE

Amanda Ardente, DVM, PhD[1,2]*, Scott Williams, MS[1], Natalie Mylniczenko, DVM, Dipl. ACZM[1], John Dickson[1] Alisha Fredrickson[1], Christy Macdonald[1], Forrest Young, MS[3], Kathleen Sullivan, PhD[1], Shannon Livingston, MSc[1], James Colee[4], Eduardo Valdes, PhD [1,2,5,6]

[1]Department of Animal Health, Disney's Animals, Science, and Environment, 1180 N. Savannah Circle, Bay Lake, FL 32830, USA
[2]Department of Animal Sciences, PO Box 110910, University of Florida, Gainesville, FL 32611, USA
[3]Dynasty Marine Associates, Inc., 10602 7th Avenue Gulf, Marathon, FL 33050, USA
[4]Statistics, Institute of Food and Agricultural Science, University of Florida, Gainesville, FL 32608, USA
[5]University of Guelph, 50 Stone Road East, Guelph, Ontario, N1G 2W1, Canada
[6]University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816, USA

Abstract
Green moray eels (*Gymnothorax funebris*) under human care are reported to have elevated plasma cholesterol and triglyceride concentrations with associated development of lipid keratopathy (Clode *et al*., 2012). Nevertheless, serum trace mineral and vitamin analyses have not been assessed, and the complete nutrient content (cholesterol, vitamins, and minerals) of managed eel diets has also not been reported (Clode *et al*., 2012; Greenwell & Vainisi, 1994). Serum biochemical, trace mineral, and vitamin A and E analyses were performed for three green moray eels managed by Disney's The Seas® and 13 recently captured, fasted, free-ranging green morays. Complete nutrient analysis was performed for managed eel diet items, and metabolizable energy was calculated (Smith, 1980). Serum cholesterol, calcium, phosphorous, iron, and vitamin E concentrations were greater ($P < 0.05$) in managed versus free-ranging eels. Serum cholesterol and vitamin E positively correlated to body weight ($P < 0.01$). Both eel populations had greater concentrations of serum iodine and lower concentrations of vitamin A when compared with other carnivorous aquatic species. Atlantic herring (*Clupea harengus*) had the greatest metabolizable energy, crude fat, and iron content but the lowest cholesterol content compared to capelin (*Mallotus villosus*) and Ilex squid (*Ilex illecebrosus*). Squid had the lowest metabolizable energy and crude fat content but greatest cholesterol content. The vitamin supplement (Mazuri® Vita-Zu Small Bird Tablet, no Vit A added 5TLC, PMI Nutrition International LLC, Saint Louis, MO 63108) provides 12g vitamin E/100g 'As-Fed' to the managed eel diet. The diet likely contributes to the development of hypercholesterolemia and influences other serum indicators of health in eels under human care. Furthermore, the crude fat content of diet items cannot be used to predict cholesterol concentration.

Acknowledgements
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for obtaining free-ranging eel blood samples and the staff at Marathon Veterinary Hospital for sample processing.

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THE INFLUENCE OF GIRAFFE BEHAVIOR ON PARASITE LOAD: IMPACT OF HUSBANDRY MODIFICATIONS AT BUSCH GARDENS TAMPA BAY

Nancy Ann-Marie Arnold, BS[1], A. Conner Cain, BS*, Robin Rowland, BS, Samantha Steele, PSM, Cara Martel, MS[2], Michael Burton, VMD[2], and Heidi Bissell, PhD[2]

[1]Department of Health Professions, University of South Florida, 4202 E. Fowler Ave., Tampa FL 33620, USA
[2]Department of Zoological Operations, Busch Gardens Tampa Bay, 3605 E. Bougainvillea Ave, Tampa, FL 33612, USA

Abstract
Haemonchus contortus is a gastrointestinal parasite that lives in the abomasal mucosa of ruminants. Similar to cattle, giraffe housed in warm climates are prone to parasitism by stronglyes such as *Haemonchus contortus*. At Busch Gardens in Tampa Bay, Florida a behavioral study and retrospective parasite survey was conducted to determine if a correlation exists between giraffe behavior, feeding methods, and parasite load. Fifteen giraffe (*Giraffa camelopardalis*) were observed to determine the proportion of observations they spent engaged in risky behaviors (i.e., grazing on the ground) vs. non-risky behaviors (i.e., feeding from elevated feeders). The fecal egg count and proportion of risky behaviors were strongly correlated. However, efforts to alter the proportion of risky behaviors by feeding more browse in a variety of elevated feeders were unsuccessful in changing the overall proportion of risky behaviors. Other techniques to discourage risky behaviors and encourage safe feeding behaviors need to be explored.

Introduction
Giraffe housed in warm climates are prone to parasitism by *Haemonchus contortus*, a gastrointestinal parasite that lives in the abomasal mucosa of ruminants. The parasite causes anemia, edema, and even death in affected animals. *H. contortus* thrives in grassy areas in tropical and subtropical climates. *H. contortus* eggs are excreted in animal feces and can live for up to two weeks without a host. Once the parasite reaches the infective stage (L3), it moves from the feces to the grass (Khatun *et al*., 2013). Normally browsers such as giraffe would rarely encounter these parasites, which require consumption of these grass-bound larvae near ground-level. However, in the predator-free environment of managed care, many giraffe will lie down on the ground or bend down to graze from the ground, putting them in proximity of these nematodes.

Medicinal anthelmintic treatments can be effective, but like antibiotics, they lose effectiveness over time as parasites become resistant (Garretson *et al*., 2009), and few new drugs are being developed to replace them. The use of copper wire particles has been attempted and found promising (Moscona, 2013) but does not completely eradicate the problem. Non-drug-based methods have been used to control parasites in a number of domestic animals. However, these techniques have varying applicability in the zoological setting. Rotational grazing requires a system of multiple pastures, typically with some remaining empty for periods of time. Few zoological facilities have spare pastures or the ability to maintain empty enclosures. Culling highly parasitized animals is likewise not considered an option in most facilities, although temporarily relocating heavily parasitized animals to grass-free enclosures is a similar technique that has been used at our institution and others (Garretson *et al*., 2009).

Other approaches include removing feces daily (scooping or vacuuming), sterilizing the soil with steam, and housing animals on gravel or other substrate rather than grassy exhibits. Finally, husbandry modifications that discourage grazing and encourage elevated feeding have potential. The objective of this study was to determine if ground-directed giraffe behaviors are related to parasite load, and if so, if we could alter giraffe behavior to minimize these potentially problematic encounters between parasites and hosts.

Materials & Methods

Subjects

Subjects included 2 male and 14 female (2015) and 1 male and 13 female (2016) and 12 female (2016 browse feeding trials) reticulated giraffe (*Giraffa camelopardalis*) housed at Busch Gardens Tampa Bay, Florida. The giraffe are housed on a 27-acre veldt which they share with zebra, ostrich, impala, addax, and eland. The veldt consists of mixed grass species, palm trees, and elevated feeders for hay and pelleted feeds. A dirt track winds through the area in a loop. During the day, guided groups of park guests tour the area in the back of flatbed trucks every 30-60 minutes and offer lettuce to the giraffe. The giraffe are fed a combination of Mazuri® Wild Herbivore Plus #5Z8W (PMI Nutrition International LLC, Saint Louis, MO 63108) and alfalfa hay along with romaine lettuce offered by guests.

Feeding behavior observations

Giraffe were observed for 60 hours from June 2015 to August 2015. Data were collected between the hours of 07:30 to 16:00, weather permitting. Between the hours of 07:30 to 09:00 data were collected from the cab of a pickup truck driving slowly along the dirt track in multiple large loops. After 09:30, data were collected in 30 minute blocks from large safari vehicles that were taking guests on tours.

Scan sampling was used for both types of data collection. From 07:30 to 09:00 behavior was recorded once per circuit as the observers drove past the giraffe. Behavior was recorded for each giraffe seen each time the truck completed a loop on the track. A giraffe identification book was used to identify giraffe via characteristics such as chest patterns. If there was any question about the giraffe's identification, then the behavior was not recorded.

All behaviors are defined and listed on the giraffe behavior ethogram (Table 1). Behaviors with a high likelihood of causing contact with ground-dwelling parasite larvae were categorized as *Risky* and included items such as *Graze Stand* (eating grass while standing, Figure 1), *Graze Rest* (eating grass while lying down), and *Debris* (eating or playing with items that had fallen to the ground). Behaviors with a low likelihood of causing contact with parasites on the ground were categorized as *Safe* and included items such as *Elevated Feed*, *Guest* (feeding on lettuce offered by guests), and *Tree Lick* (licking trees). Two behaviors could arguably be categorized either way. *Graze Wall* was recorded when the animals grazed on an elevated wall with grass growing on top. This grass was considered a low parasite risk because no animal feces would be up so high to incubate the parasite. Therefore, *Graze Wall* was not considered a *Risky* behavior, even though it involved consuming grass. *Rest* (lying down while not eating) is unlikely to lead directly to consuming parasites. However, it does increase contact with the ground, and animals that are resting have both the parasite larvae and grass close by. This presumably increases their likelihood of consuming them. For these reasons, it was included in the *Risky* category.

Fecal parasite monitoring

Fecal samples from each giraffe are collected twice monthly as part of the herd's routine monitoring program. Egg counts (derived using the McMaster egg counting technique; Coles *et al*., 1992), anthelmintic treatments, dates, and dose were obtained from each animal's medical record from August 2012-August 2016. Egg counts were then averaged by animal by month of year, quarterly season (Winter: December-February, Spring: March-May, Summer: June-August, and Fall: September-November), and climate season (rainy season: May-October, dry season: Nov-April), and overall.

Browse feeding observations

During the summer of 2016, browse was provided to the animals at randomized times within three time slots, morning, noon, and evening. On evenly numbered dates (e.g., July 14 and 16), browse was put out at noon and during an evening time, and on odd numbered dates (e.g., July 15 and 17), browse was put out in the morning and at noon. Observations sessions lasted for one hour after browse was installed. On average, browse made up 35% of total weekly offered giraffe diet (± 3.2% SD). Browse was not put out if lightning was reported within five miles during the length of a time slot due to safety protocols. Browse was put out every day during the study period. Observations were only conducted on weekdays, with the exception of one weekend.

Browse was offered using four different methods which were rotated biweekly. In the *hayrack* option (n = 19, Figure 2a), browse was put into tall standalone hayrack on top of whatever hay was left over from earlier feedings. Only one hayrack was used per observation. In the *lattice* option (n = 21, Figure 2b, browse was woven through and stuffed in between two plastic lattice fence pieces cut into roughly 4.5x4 foot rectangles and zip-tied together on all sides but the top to form a pocket. Each lattice pocket was then clipped together on the top and clipped to eyehooks drilled into trees on the veldt. Up to four lattice structures could be hanging during a single observation. During the *mix* option (n = 19), browse was put into the hayrack and at least one lattice was installed during the same observation session. Up to 4 sources of browse could be available during a mix observation. The *velcro* option (n = 5, Figure 2c) was only put out whenever large tree limbs were available and was only possible during 5 observations. When possible, large sticks (over 1 inch in diameter) were wrapped up in a long strap into a bundle which was velcroed together, and the bundle was attached with a clip to a tree eyehook. Multiple bundles could be put up on multiple trees during one observation session. During each observation session, the lattice and velcro feeders could be installed in three potential locations, which were randomly chosen each session. Observers categorized giraffe-browse interactions into four distinct behaviors: *Eating*, *Investigate*, *Take Out*, or *No Involvement* (Table 1) and recorded the individual giraffe, the browse species, and the duration of the behavior by noting start and end times for the behavior. Eating and Take Out were categorized as *Engaged* with the browse, while Investigate and No Involvement were *Unengaged*.

The data were analyzed using ANOVA to determine the relationship between each animal's proportion of behaviors that were *Risky* with their fecal egg counts measured two ways: the egg count at the time of observation (Instant Egg) and a single 4-year average (Average Egg) for each animal. The impact of offering browse (any feeding method) on proportion of *Risky* behaviors as well as the impact of browse feeder type on the time spent engaged with the browse was also measured using ANOVA in R (Version 3.3.2, Vienna, Austria).

Results

There was a strong, positive, linear correlation between percentage of observations with *Risky* behaviors and average egg count ($df(1,19) = 6.74$, $P < 0.018$, Figure 3) as well as the instant egg count ($df(1,19) = 6.92$, $P < 0.017$, Figure 1). Egg counts did not vary by month, quarterly season, or year. However, egg counts were higher during Florida's rainy season (May-October) than during the dry season (November-April; $df(1,786) = 3.87$, $P < 0.05$, Figure 4).

During the browse feeding trials, there was no difference among the four feeding methods in either the amount of time the giraffe spent *Eating* or *Engaged* or the total number of giraffe present during that session ($P > 0.5$). In addition, neither the presence of browse (any presentation) nor any single type of feeder affected the proportion of observations spent engaged in *Risky* behaviors. While some individual giraffe increased the proportion of *Risky* behaviors with the browse provision, others decreased (Figure5).

Only a small fraction of the giraffe herd approached the browse feeders during the observations. However, other individuals may have fed from them after the period had ended. The type of elevated feeder was not significant, but the specific species of browse provided did have an impact on the number of giraffe feeding from the feeders and was likely a significant source of variation in this study. Some browse species such as tipuana (*Tipuana tipu*), cut grasses, acacia (*Acacia auriculiformis*), and willow (*Salix carolinensis*) were highly preferred. When these species were offered, the amount of time the giraffe herd was engaged with the feeders and the number of giraffe engaged increased.

Discussion

There was a strong correlation between the amount of time spent doing risky behaviors and parasite load. Although our data are merely correlations and do not establish causation, the information we have about how the parasite is transmitted indicates that it is highly likely that increased contact with the ground increases the chances of having a high parasite load. As such, minimizing *Risky* behaviors is a priority, especially when partnered with medical treatments such as anthelmintic drugs, copper wire, and pasture management techniques.

The four different behavioral modifications (different types of elevated feeders) were not universally accepted by the animals, and even for the animals that did engage with the feeders, they did not alter their overall proportions of risky behaviors. Thus, providing giraffe with browse and/or elevated feeders may not be sufficient to alter their overall behavioral patterns and reduce the risk of parasite infections. Even preferred browse species were not able alter the behavioral patterns observed.

Conclusions

Based on this study, behaviors that put giraffe in contact with the ground likely play a strong role in determining the extent of their parasite load. However, feeding browse in elevated feeders twice a day did not change the giraffes' feeding behaviors sufficiently enough to cause a change in the proportion of these risky behaviors or their parasite load. In a positive-reinforcement environment, desired foods such as hay, pellets, and browse are some of the strongest tools to encourage giraffe to engage away from the ground. However, this may not be sufficient to deter ground-directed

behaviors. Other ways of encouraging safe, elevated behaviors should be investigated beyond just elevated feeders, and other non-behavioral means to reduce parasitic infections should be considered as well.

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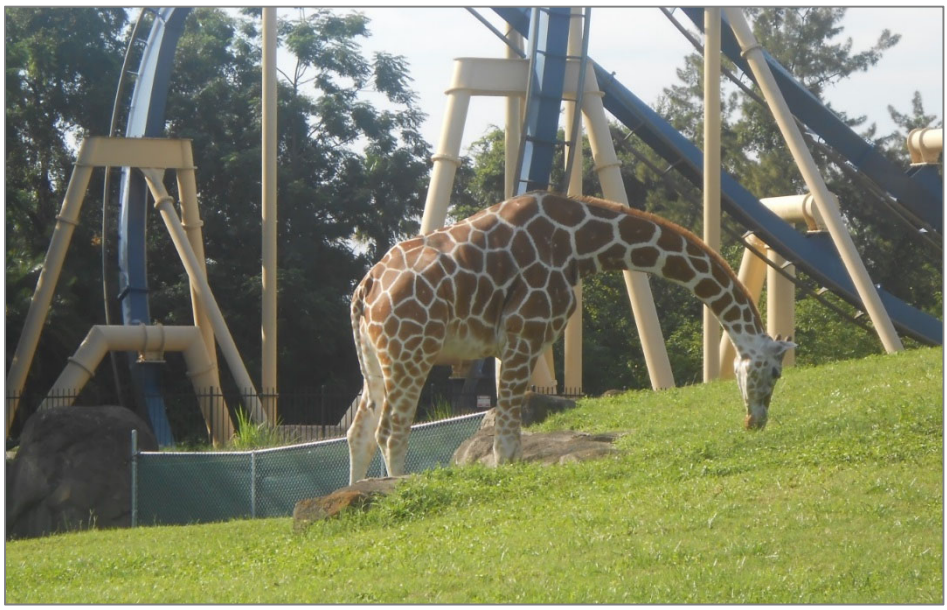

Fugure 1. A giraffe (*Giraffa camelopardalis*) at Busch Gardens Tampa Bay grazing on grass on the ground, a behavior associated with increased risk for parasites.

Table 1. Definitions (ethogram) of giraffe (*Giraffa camelopardalis*) behaviors recorded in current study

Risky: ground-directed behaviors with a high likelihood of parasite transmission (2015 and 2016)	
Debris	Giraffe is eating food, palm fronds or sticks that have fallen to the ground.
Graze Rest	The giraffe is lying on the ground with legs under or close to body with its head lowered and eating grass from ground.
Graze Stand	The giraffe is standing with its head lowered eating grass from ground
Rest	The giraffe is lying down with legs bent and tucked under or close to body on the ground.
Safe: Behaviors not directed at the ground or objects on the ground, low likelihood of parasite transmission (2015 and 2016)	
Elevated Feed	Giraffe eats from the elevated feeders or tree tops with raised head. Does not include eating fallen debris e.g., palm fronds.
Graze Wall	The giraffe is eating grass from an elevated location (wall).
Guest	Giraffe eats romaine lettuce from guest.
Observed	Animal was observed doing something other than the behaviors above.
Tree Lick	Giraffe licks tree bark
Browse-feeding behaviors (2016 only)	
Engaged with browse	
Eating	Engaging browse with mouth within 2 feet of browse, including chewing and licking
Take out	Eating branch or browse taken from browse source more than 2 feet away from source
Unengaged with browse	
Investigate	Giraffe within 5 feet of browse, but not engaged with *Eating* or *Take out*
No involvement	Animal did approach browse or within 5 feet of browse feeders

A. Hayrack

B. Velcro

C. Lattice

Figure 2. Browse feeding treatments used in the current study. In the *hayrack* option, browse was put into tall standalone hayrack on top of whatever hay was left over from earlier feedings. In the *lattice* option, browse was woven through and stuffed in between two plastic lattice fence pieces cut into roughly 4.5x4 foot rectangles and zip-tied together on all sides but the top to form a pocket. Each lattice pocket was then clipped together on the top and clipped to eyehooks drilled into trees on the veldt. In the *velcro* option, large sticks (over 1 inch in diameter) were wrapped up in a long strap into a bundle which was tied together with velcro straps, and the bundle was attached with a clip to a tree eyehook.

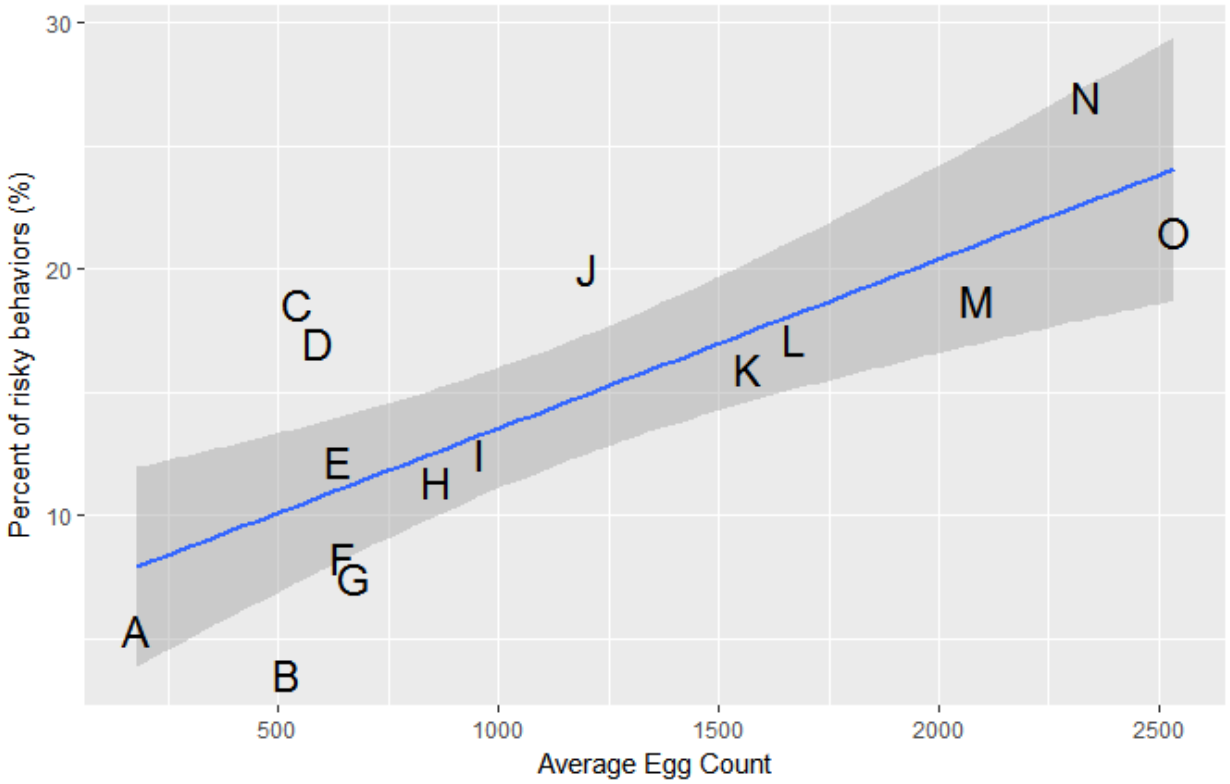

Figure 3. Correlation between fecal egg counts and the percentage of observations giraffe (*Giraffa camelopardalis*) performed risky behaviors, such as grazing on the ground. Letters represent individual giraffe.

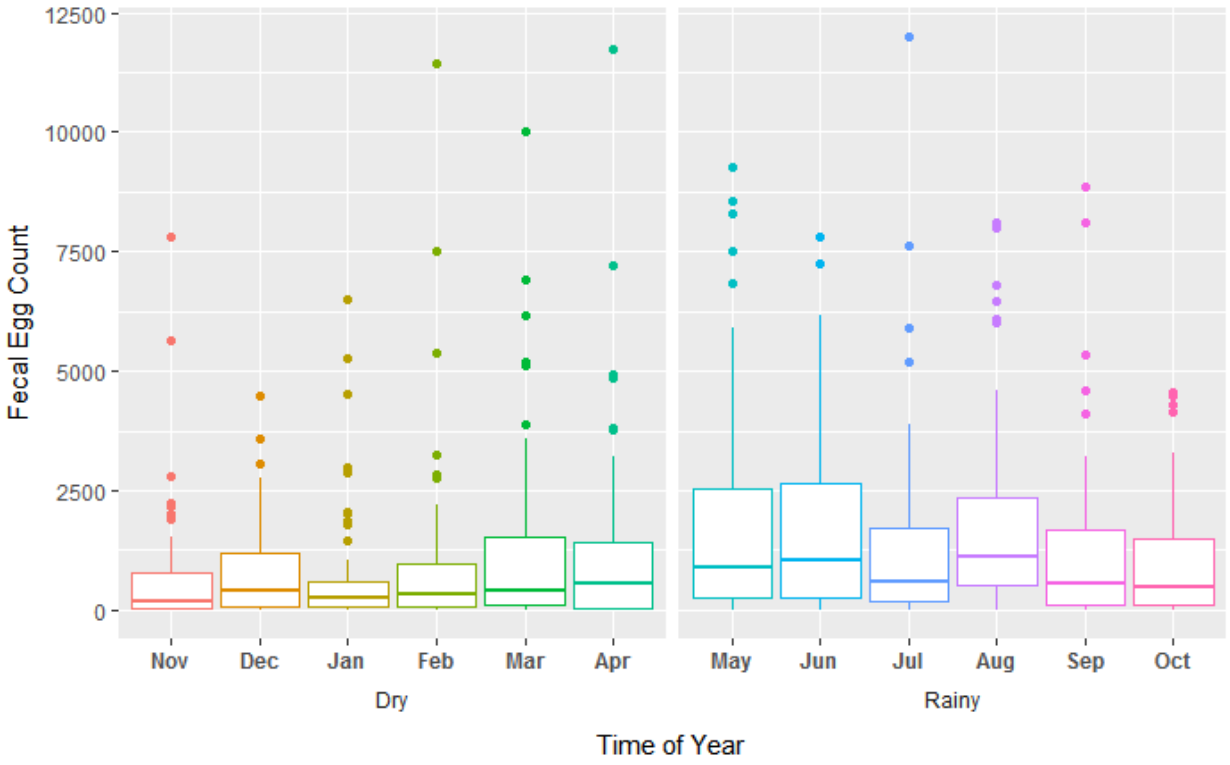

Figure 4. Giraffe (*Giraffa camelopardalis*) fecal egg counts during the dry season and rainy season in Florida (Four values > 12,500 eggs not shown. Three of these were in the rainy season, one in the dry).

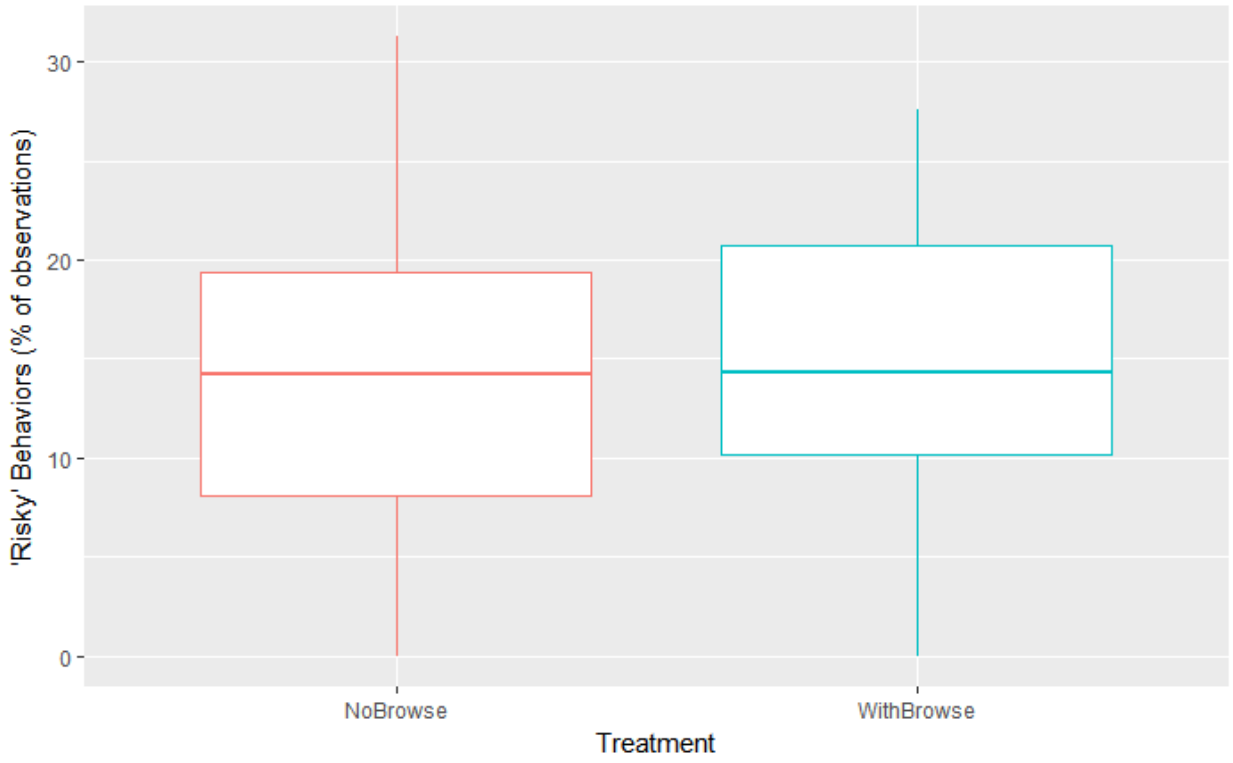

Figure 5. The proportion of risky behaviors performed by giraffe (*Giraffa camelopardalis*).

A SIMPLE, PRACTICAL METHOD FOR MEASUREMENT OF FAT IN MILK, APPLIED TO SAMPLES FROM MID- TO LATE-LACTATING WORKING ELEPHANTS IN A MATERNITY CAMP IN MYANMAR

Yadana Aung Myo Han, BVS, VMSc[1,2], Ellen S. Dierenfeld, BS, MS, PhD[3]*, Khyne U. Mar, BVS, MSc, PhD, FRVCS[4], Mirkka Lahdenperä, MSc, PhD[5], Virpi Lummaa, MSc, PhD[5], Aung Aung, BVS, PhD[2]

[1]Department of Medical Research (Pyin-Oo-Lwin Branch), Pyin-Oo-Lwin, Myanmar
[2]Department of Physiology & Biochemistry, University of Veterinary Science, Yezin, Myanmar
[3]Ellen S. Dierenfeld, LLC, St. Louis, MO USA
[4]Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK
[5]Section of Ecology, Department of Biology, University of Turku, FIN-20014, Turku, Finland

Introduction

During late pregnancy and throughout lactation, logging elephants owned by Myanmar Timber Enterprises are managed separately from the working herd in select maternity camps, affording training, observation, and research opportunities that target improved management, health, and calf survival (http://myanmar-timber-elephant.group.shef.ac.uk/). This project was undertaken as part of a larger investigation to examine relationships among nutrient composition of milk, forages consumed by the cows, and growth/health response of elephant calves. Although elephants have been trained for manual milk collection, potential challenges to standardized laboratory analysis for milk in Myanmar include sample size, transportation, limitations of reagents, equipment, and electricity, as well as applicability of methodologies developed for dairy livestock species to wildlife. Here we describe a modification of a rapid, economic method that has previously been used for estimating fat and energy content in livestock (Fleet and Linzell, 1964) and human (Lucas *et al*., 1978) milks, tested on elephant milk samples, for ultimate field application.

Methods

To test the methodology, fresh milk samples from local dairy cows in Myanmar and purchased whipping cream were thoroughly mixed, and ~75 µl aliquots ($n = 8$) were drawn by capillary action into standard glass capillary tubes (75 X 15 mm outside diameter) that were sealed by clay. Tubes were centrifuged at 12,000 rpm (approximately 14,500 x *g*) for 15 min using a hematocrit centrifuge (Clay Adams Autocrit model CT-2905, New York, NY USA), then immediately the fat layer(s) at the top were measured to the nearest 0.01 mm using digital calipers (Powerfix model Z22855, Paget Trading Ltd., London, UK), and expressed as a percentage of the total milk column, following the details in Lucas *et al*. (1978). Measurement of the solid fat layer meniscus was measured at the top, rather than bottom due to opacity. If clear, liquid fat was present at the top of the cream layer, that fraction was also included with the fat measurement. Measured values compared favorably with known crude fat content of dairy products in Myanmar (3.39 to 4.25% for milk; 56.67% for whipping cream).

Milk samples (5 to 20 ml) from 6 Asian elephant (*Elephas maximus*) cows were obtained on 6 separate dates between the wet season (Jul through Sep 2016; $n = 3$) and dry season (Oct 2016 through Mar 2017; $n = 3$) from mature cows in mid- (16 mo) to late lactation (38 mo; see Table 1 for animal details). Aliquots were blended by inversion and measured in duplicate to obtain

"creamatocrit" and liquid fat percentages as per the protocol described above. Percent solids, crude protein, ash, and vitamin E were also determined on the elephant milk samples using published methods (AOAC, 1990); values are reported elsewhere (Han, 2017). Descriptive statistics, paired *t*-comparisons of means (wet vs. dry season), and one-way ANOVA were conducted using SPSS (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

Results

One sample was determined to be an outlier and not used in further analyses. Centrifuged elephant milk samples separated into 3 distinct layers: the bottom aqueous fraction, presumably containing proteins, carbohydrates, and soluble minerals; a dense white fat layer; and a clear liquid lipid layer (Figure 1). Percentage lipid fractions in Myanmar elephant milk samples, using the simplified "creamatocrit" method, are found in Table 2 (15.35 mean $\pm$ 4.44 SD %). The liquid fat layer accounted for (78.88 ± 12.52%, range 30.99% to 97.14%) of the total fat, as opposed to the denser cream layer (21.11 ± 12.52%; range 2.86% to 69.01%). The large liquid fat layer in elephant milk differs substantially from results using this method with human or bovine milk samples, where the liquid layer was non-existent or comprised a minor proportion (Lucas *et al*., 1978). Total fat content ranged from 7.20% to 26.47% in milk samples over the 9 months of this sampling period. Significant effects of individual animal, associated with stage of lactation, were found (Table 3); cow age and/or parity effects were not statistically significant. Seasonality was also not significant in this dataset; the mean wet season fat values (15.57 ± 1.47%; $n = 17$ samples from 6 cows) were identical to values measured through the dry season (13.52 ± 3.29%, $n = 18$ samples from 6 cows). However, if lag effects due to diet difference are considered, fat content of milk samples from the beginning of the dry season (end of wet season, October; 17.37 ± 2.05%) differed significantly from samples collected at the end of the dry season 12.77 ± 3.05%; $P < 0.01$ (Figure 2).

Discussion

Despite the use of standardized methodologies for proximate analysis of milk samples, high fat content of the elephant milk samples resulted in a non-quantifiable liquid fat residue regardless of time in the drying oven to determine water content/dry matter, hence, proximate fractions quantified do not necessarily correspond with total solids measured. Due to limitations of sample sizes and in-country laboratory capabilities, we evaluated total fat content using this low-technology, practical method that could also be transferred to the field for immediate monitoring of milk quality and potential nutritional interventions, if necessary.

While overall mean total fat values (15.35 ± 4.44%) in our study average considerably higher than those reported by Mainka *et al*. of 7.6 ± 2.6% (1994; $n = 1$ cow) during the first 9 mo of lactation, Simon (1959) and Peters *et al*. (1972) reported levels varying from 0.95% to 19.0% during the first 18 mo of lactation. More recently, Abbondanza *et al*. (2013) demonstrated distinct changes in milk fat content throughout lactation in the same individuals ($n = 3$ cows), following the same pattern as reported for African elephants by McCullagh and Widdowson (1970). For captive-fed Asian elephants, milk samples taken at $\leq$ 9 mo of lactation contained < ~13% fat, whereas samples taken from 18 to 30 mo of lactation contained 15-20% fat. As the Myanmar samples were all from mid- to late-lactation cows, our data fit well with these described patterns and confirm high fat content in late lactation milks from Asian elephants. In general, the protein and fat contents of elephants' milk increase over lactation time.

Although stage of lactation impacted milk fat content in logging camp elephants, there was no significant effect of seasonality in this data set if one defines seasons strictly by calendar months. Nonetheless, milk fat decreased significantly from the beginning to the end of the dry season, with individual cows (4 of 6) displaying end of season fat values ~60 to 70% those seen 5 months earlier, likely due to nutritive changes in available vegetation (data not shown here) and/or hydration status of the animals. In the case of the latter, however, one might expect milk fat to increase or not change, since higher milk fat has been considered a physiologic mechanism for water conservation in elephants (Abbondanza *et al*., 2013). Consistent seasonal (environmental) effects and potential impacts on nursing calves need to be examined through continued long-term monitoring programs of the elephant herds.

McCullagh and Widdowson (1970) previously reported that elephant (African; *Loxodonta africana*) milk lipid globules are half the size of bovine milk fats, with a unique fatty acid signature; fatty acid details of Asian elephant milk have not been published. Osthoff *et al*. (2007) confirmed a high content (~70%) of short-chain saturated fatty acids (FAs), particularly capric and lauric acids, low levels of polyunsaturated FAs, and omega-3: omega-6 FA ratios of approximately 1:1 in mid-lactation African elephant milk, with increasing degree of short chain FA as lactation progresses. Regulatory mechanisms for this process are not yet defined, nor have they been examined in Asian elephant milks. While smaller and shorter chain lipid molecules may be more liquid at room temperatures, the observation of widely ranging proportions of both liquid and solid fat fractions in the centrifuged elephant milk samples need to be investigated further. It is tempting to speculate that the highest proportion of liquid lipid fractions, found in samples from the 2 cows with the oldest calves, may represent this pattern of increased short chain FA in later lactation milks, but no clear pattern of variation (by individual, stage of lactation, month, or season) was discernable in this limited data set.

Most importantly, however, this study documents that reliable fat values for milk samples can be quantified rapidly and economically using this methodology. In the field, it can be very difficult to collect both the samples, as well as substantial quantities for analysis. By using the small volume necessary for this technique, we can quickly assess dam milk fat quality and calculate energy values, while minimizing removal of essential nutrients for the growing calf.

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Table 1. Description of Asian elephant cows (n = 6) in a Myanmar maternity camp from which milk samples were obtained for nutritional analysis between 18 and 30 mo of lactation.

No.	Name of elephant	Age (year)	Parity No.	Body Wt. (kg)	Date of Calf Birth	Sex of baby
1	Than Bo Mi	40	2	2964	23.03.2015	Female
2	Swe Htay Oo	41	4	2358	02.01.2015	Female
3	Htoo Thin Kyi	35	2	2407	27.02.2015	Male
4	Thin Aye Moe	26	3	2582	16.01.2014	Male
5	Shu Zarni	24	2	2404	30.06.2014	Male
6	Win Moe Thwe	31	2	2706	22.09.2014	Male

Table 2. Percentage of total milk fat in Asian elephant milk samples, measured using the "creamatocrit" methodology; both solid and liquid lipid fractions were apparent in all samples.

Description	Unit	*n*	Minimum	Maximum	Mean	Std. Deviation
Total Fat	%	35	7.20	26.47	15.35	4.44
Cream	% Total Fat	35	2.86	69.01	21.11	12.52
Liquid	% Total Fat	35	30.99	97.14	78.88	12.52

Table 3. Variation of milk fat composition from individual Asian elephants in Myanmar, sampled between 16 and 38 months of lactation.

	Animal					
	1	2	3	4	5	6
	Mo of Lactation					
Parameter	**16 - 24**	**18-25**	**17-25**	**30-38**	**25-33**	**22-30**
Milk fat, %	14.56±4.78	16.14 ± 2.55	15.63 ± 7.08	12.73 ± 3.44	16.23 ± 2.90	15.36 ± 4.96
% Cream fat	15.93^{d}	33.97^{a}	29.52^{b}	17.93^{d}	22.54^{c}	14.9^{d}
% Liquid fat	85.07^{a}	71.36^{b}	72.48^{b}	79.40^{ab}	85.90^{a}	87.93^{a}

^{abcd}Values with similar superscripts within the same row are similar ($P \leq 0.001$)

Figure 1. Measuring milk fat in elephant milk samples (as a percentage of total sample) using a simplified centrifugation technique and calipers, suitable for field sampling.

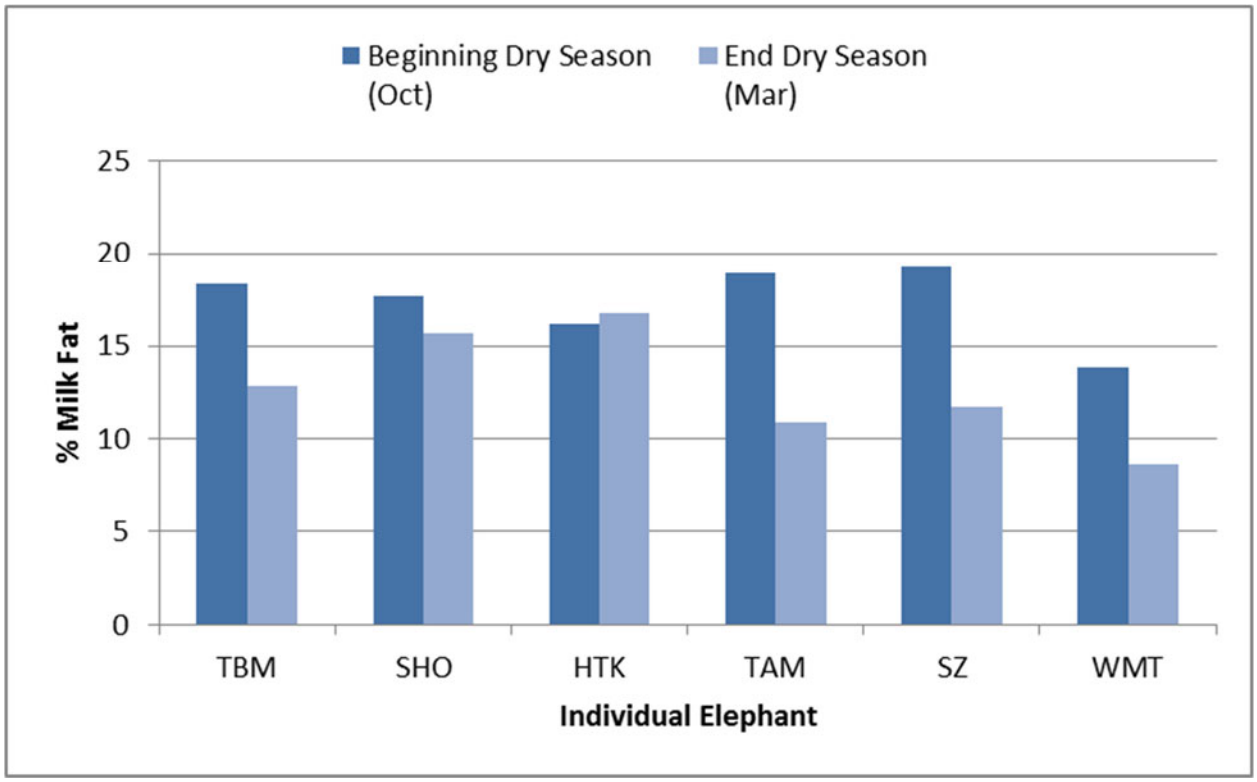

Figure 2. Total milk fat percentages from semi-free ranging Asian elephants (n = 6) in Myanmar sampled at beginning (Sep/Oct 2016) and end of the dry season (Mar 2017), between 19 and 38 months of lactation.

MACRONUTRIENT SELECTION IN MAMMALIAN INSECTIVORES AT BUSCH GARDENS TAMPA BAY

Heidi Bissell, PhD

Department of Zoological Operations, Busch Gardens Tampa Bay, 3605 E. Bougainvillea Ave, Tampa, FL 33612, USA

Abstract

This study determined macronutrient target of several mammalian insectivore species including 3-banded armadillo (*Tolypeutes tricinctus*), aardvark (*Orycteropus afer*), tamandua (lesser anteater) (*Tamandua tetradactyla*), and greater anteater (*Myrmecophaga tridactyla*). These insectivores are often maintained on a commercial insectivore diet, which may or may not be appropriate for every species in this diverse and polyphyletic group. Animals were fed three experimental diets that varied in their proportions of calories from protein, fat, and carbohydrate. Quantities of each diet consumed were measured, and the total amount of calories consumed of protein, fat and carbohydrate were calculated. Results indicate that the different species may have different macronutrient targets, with the generalists (armadillos) selecting higher carbohydrate diets than the termite- and ant-eating specialists. This indicates that these groups may have different evolutionary targets and perhaps also different nutrient requirements as well. A larger sample size is needed.

ANALYSIS OF FECAL GLUCOCORTICOID CONCENTRATION IN AFRICAN (*LOXODONTA AFRICANA*) AND ASIAN (*ELEPHAS MAXIMUS*) ELEPHANTS IN RELATION TO MANAGEMENT AND NUTRITIONAL FACTORS

Jessica D. Bray, MS[1]*, Kimberly Ange-van Heugten, PhD[1], David Dickey, PhD[2], Charlotte Farin, PhD[1], Kathy Carlstead, PhD[3], and Janine Brown, PhD[4]

[1]Department of Animal Science, North Carolina State University, PO Box 7621, Raleigh, NC 27695-7621, USA
[2]Department of Statistics, North Carolina State University, 5109 SAS Hall, Raleigh, NC 27695, USA
[3]Portland, OR, USA
[4]Smithsonian Conservation Biology Institute, 1500 Remount Dr., Front Royal, VA 22630, USA

Abstract

Identifying relationships between management and nutrition factors related to the physiological needs of elephants under human care is vital for improving husbandry and welfare standards. This study consisted of 130 African (*Loxodonta africana*) and 104 Asian (*Elephas maximus*) elephants housed in 65 separate facilities throughout North America. Fecal samples were obtained every other week for 12 months and analyzed for fecal glucocorticoid metabolite (FGM) concentrations by EIA. All management and housing data were collected by electronic survey methods. Repeated measures analyses were run to identify relationships between FGM and sex, environment (climate zone and season), and management (enrichment, animal contact, feed total, spatial experience, herd size, and other social group factors) factors. Analysis showed that African elephants had higher FGM concentrations at facilities where animals were fed more frequently ($P < 0.05$). In addition, as the number of different social groupings an African elephant experienced at their facility increased, there was a positive correlation to FGM concentrations ($P < 0.05$). However, as the number of elephants an individual had contact with during the day increased, significant decreases in average FGM level were exhibited by those individuals amongst the African population. In contrast, these same relationships were not duplicated for the captive Asian elephant population. Therefore, further investigation into differences in diet management and associated social interactions amongst the facilities may need to be conducted. These results may impact the future of husbandry and management practices related to captive elephant overall health and welfare.

GUT-LOADING DIET EVALUATION FOR CRICKETS (*ACHETA DOMESTICUS*), MEALWORMS (*TENEBRIO MOLITOR*), AND SUPERWORMS (*ZOPHOBAS MORIO*) FOR THE PURPOSES OF OPTIMIZING INSTITUTIONAL PROTOCOLS

Matthew A. Brooks, PhD* and Gwen I. Harris, MA

Living Collections, Oregon Zoo, 4001 SW Canyon Rd., Portland, OR 97221, USA

Abstract

The Oregon Zoo recently experienced multiple cases in our herpetology and bird departments of hypovitaminosis A and possible metabolic bone disease that prompted an evaluation of current insect gut-loading protocols and feeds. Two trials were conducted with crickets, mealworms, and superworms to evaluate the effectiveness of different gut-loading diets to increase Ca:P ratios and vitamin A concentrations in these feeder insects. Trial 1 evaluated the proximate and mineral analysis of insects fed a commercially produced bird starter diet, two different commercially produced insect gut-loading diets, and one in-house chopped diet mix. Trial 2 evaluated the vitamin A concentration of the three different commercially produced diets. Results indicated the bird starter diet and in-house diet mix did not produce adequate Ca:P ratios in the feeder insects. However, the two gut-loading diets had varying effectiveness on Ca:P ratios due to insect species. Further, vitamin A levels were not increased based on the diets offered, with the crickets and superworms having concentrations below detectable limits. One issue seen with one of the commercial insect gut-loading diets was an increased mortality of the superworms and mealworms. From the study, a diet was chosen based on Ca:P ratios and survivability of insects on the diet.

Introduction

Several papers over recent years have looked at the practice of gut loading feeder insects for the purposes of improving nutrient composition of these diet items for an array of insectivores; most important being amphibians, reptiles, and birds (Attard, 2011; Coslik *et al*., 2009; Hunt *et al*., 2001). These particular species are especially prone to nutritional maladies including metabolic bone disease, rickets, osteomalacia, and hypovitaminosis A (McWilliams and Leeson, 2001; Pessier and Rodriguez, 2015). The Oregon Zoo recently experienced multiple cases in our herpetology and bird departments that necessitated an evaluation of gut loading protocols throughout the zoo. These include presentation of hypovitaminosis A in a rough-skinned newt, a ruddy duck, and hammerkop chicks, and possible metabolic bone disease in a Meller's chameleon and red-billed hornbill chick.

After evaluating the protocols, it was determined that the departments were using different methods for gut loading their feeder insects. To help with consistency and quality control of the feeder insects, and to evaluate the differences between newer and established gut loading diets, a series of trials were conducted to address the animal health concerns and keeper concerns. One of the chief concerns brought by the keeper staff was the increased insect mortality on a newer gut loading diet compared to our previous established gut loading diet. Second, was the concern regarding vitamin A content of the gut loading diet, and third was the overall effectiveness of a gut loading versus the time and effort required manage multiple insect containers in an area.

The first objective of this study was to determine the effect on mortality of how the gut loading diet is presented. The second objective was to determine the efficacy of different gut loading diets on increasing the Ca:P ratio in feeder insects. The third objective was to determine the efficacy of different gut loading diets on increasing vitamin A content on feeder insects.

Materials and Methods

Insect Receiving

All insects were obtained via a commercial distributor (Redline Food, Inc., 1990 NW Cadbury Ave. Suite 202, Beaverton, OR 97006) and shipped overnight to the Oregon Zoo. Once received, all insects were put into polycarbonate containers and provided with Purina Game Bird + Turkey Startena Diet (PS, Table 1; PMI Nutrition International LLC, Saint Louis, MO 63108) for 24 hours before being put into any of the trials. For mealworms and superworms, PS diet was provided as a bedding substrate with fresh collard greens provided as a source of moisture. For crickets, PS diet was provided in a dish with a separate dish of water provided. All trials were conducted in a climate controlled room in the Oregon Zoo Veterinary Medical Center, with the temperatures kept between 23-28°C throughout the trial.

Pre-trial mortality assessment

Prior to the gut loading trials, 1000 mealworms and 1000 superworms were divided into 3 treatment groups. Treatment groups consisted of PS offered as a bedding substrate, Mazuri® Better Bug® Gut Loading Diet #5B45 (MBB; Table 1; PMI Nutrition International LLC, Saint Louis, MO 63108) offered as a bedding substrate, and MBB offered in a food dish with no bedding substrate. The nylon bags the worms arrived in were cut up and used as covers in which the insects could hide. Insects were counted into the containers, and live and dead insects were counted out of the containers after 48 hrs.

Gut loading trial 1

The 3 species of insects were each divided into 4 treatment groups with 3 replicates per group with 250 Crickets or Superworms per replicate and 500 mealworms per replicate. Treatment groups consisted of PS, Mazuri® Hi Calcium Gut Loading Diet #5M38 (MHC; PMI Nutrition International LLC, Saint Louis, MO 63108), MBB, and our in-house Oregon Zoo Tortoise Chop (OZTC, Table 1). The OZTC consisted of 16% Bok Choy, 16% Broccoli, 12% Collard Greens, 19% Kale, 16% Shredded Carrot, and 21% Ground Mazuri® Tortoise LS Diet (all on a DM Basis; Table 1; PMI Nutrition International LLC, Saint Louis, MO 63108). All insects were provided the treatment diets *ad libitum* in a polycarbonate dish in each container for 48 hrs. Crickets were also provided a separate dish water for moisture. At the end of 48 hrs, the number of dead insects was recorded and removed. All remaining live insects were euthanized via CO_2 by our veterinary medical staff and stored at -80°C before shipping. Samples of all treatment diets were also taken during the study. At the completion of the trial, all samples were packed on dry ice and sent to Dairy One Forage Laboratory (730 Warren Rd., Ithaca, NY 14850 USA) for proximate and mineral analysis.

Gut loading trail 2

The 3 species of insects were each divided into 3 treatment groups with 3 replicates per group with 250 Crickets or Superworms per replicate and 500 mealworms per replicate. Treatment groups consisted of PS, MHC, and MBB. Cricket feeding and care was consistent with the methods used

in Trial 1 (above). At the completion of the trial, all samples, including diet samples of PS, MBB, and MHC, were packed on dry ice and sent to Nestle Purina Analytical Laboratories (824 Gratiot, St. Louis, MO 63102 USA) for vitamin A analysis.

Statistics

All samples were compared only within species. All statistical analyses were performed by single-factor ANOVA. When the *F*-test was significant ($P \leq 0.05$), means separation was performed using Tukey HSD.

Results

Pre-trial mortality

The pre-trial mortality assessment (Table 2) showed that feeding MBB as a bedding substrate instead of in a separate dish for the larval insects caused a drastic increase in the mortality for both the mealworms and superworms over the normal 48 hour gut loading time frame (30.7% and 100%, respectively).

Gut-loading trial 1

Crickets

All results from the cricket portion of this trial can be found in Table 3. As the focus of this trial was to look at the gut-loading for the purposes of Ca and P, those results will be highlighted will be highlighted in the text. Crickets in the MBB group showed greater Ca levels (0.88%, $P < 0.001$) than any of the other groups. The proportion of Ca in the MHC was almost half of the proportion found in the MBB group (0.48%), and the amounts in each of the PS and OZTC groups (0.17% and 0.12%, respectively) were less than a quarter of the amount in the MBB group. Although the amount of P was greater in the MBB group than the PS or MHC groups, with the OZTC being similar to all values ($P = 0.01$), the range of difference was not physiologically significant (0.85-0.90%). However, the Ca:P ratio was greater in the crickets on the MBB diet (0.98, $P < 0.001$) than any other diets, followed by the MHC diet (0.57), and then the similar levels seen in the PS and OZTC diets (0.20 and 0.13, respectively).

Mealworms

The Ca levels in the mealworms (Table 4) were greater in the ones fed the MHC diet (1.05%, $P < 0.001$, Table 4), with the MBB mealworms containing half the level (0.55%) of the MHC mealworms, and the PS and OZTC mealworms being similarly lower than both other groups (0.15% and 0.14%). The P levels were not different between mealworms fed any of the gut-loading diets ($P = 0.09$), but the Ca:P ratio was greatest in the MHC mealworms (1.31, $P < 0.001$), followed by the MBB group (0.70), and the PS and OZTC groups (0.19 and 0.18, respectively).

Superworms

The Ca levels in the superworms fed the MHC and MBB diets (0.23% and 0.33%, respectively; Table 5) were greater ($P < 0.001$) than the levels seen in the superworms fed the PS (0.06%) and OZTC (0.07%) diets (Table 5). The P measured in the superworms fed the PS diet were greater (0.66%, $P < 0.001$) than those measured in any of the other diets (0.57-0.58%). The Ca:P ratio was similarly greater ($P <0.001$) in the MHC and MBB diets (0.41-0.57) than the PS and OZTC diets (0.09-0.13). Also, as seen in the pre-trial mortality study, there was a high death loss of

superworms fed the MBB diet during this trial (16.34%), whereas there was no death loss seen when mealworms were fed any of the other diets in the trial.

Gut-loading trial 2

For many samples, vitamin A, measured as retinol and the esters, was below detectable levels (1.66 IU A/g DM; Table 6). None of the superworm samples had detectable vitamin A, and no vitamin A was detected in the crickets fed MHC or MBB. Crickets fed the PS diet had 1.99 IU A/g DM. Mealworms all showed low levels of vitamin A (1.74-2.14 IU A/g DM), but there was no difference due to effect of diet.

Discussion

For captive insectivorous birds, reptiles, and amphibians, gut-loading has become an essential part of a balanced diet when feeding commercial feeder insects. The captive bred crickets, mealworms, and superworms being fed have inadequate Ca and vitamin A to meet the nutritional needs of these animals. The trials conducted for this study were to determine which gut-loading diet would be the best option for our collection. The PS diet was included as the control diet. When insects are not being gut-loaded, this is the diet on which the insects are maintained. This diet is not meant to have a gut-loading level of Ca or vitamin A, as shown in Table 1. The OZTC diet was included as part of our trial diets because our herpetology department wanted a diet that contained vegetable items that were naturally high in vitamin A and its precursor, beta-carotene. There is evidence that this type of diet would increase retinol levels in toads fed crickets gut-loaded in this manner (Odum *et al.*, 2015) without an additional gut-loading diet. This diet was designed to be fed to herbivorous reptiles in our collection, and thus the Ca:P ratio was 2.02 by design. The last two diet options, MHC and MBB, were both commercially available gut-loading diets with similar Ca and vitamin A concentrations. The difference between the two comes down to protein and fat concentration and ingredient list, with the MBB containing more CP and fat coming from fishmeal and spirulina instead of the corn, soybean meal, and porcine meal in the MHC.

The results from Trial 1 (Tables 2, 3, and 4) show that both the PS and OZTC diets produced insects with very low Ca:P ratios. This showed fairly conclusively that insects produced from feeding these two diets would lead the consuming animals to Ca and P imbalances that would be detrimental to their health. The results from the MHC and MBB fed insects were species specific. In crickets, the MBB diet produced a Ca:P ratio of approximately 1, while the MHC was significantly lower at about half of the MBB levels. Conversely, the mealworms did better on the MHC diet with a more desirable Ca:P ratio of 1.31 and the MBB diet producing a ratio half the level of the MHC. The superworms Ca:P ratios were similarly about 0.5 for both MBB and MHC fed crickets, which is well below a desired 1.0 or higher. This allowed us to make the decision that our final choice would be between the MBB and MHC diets, because the OZTC and PS diets would never be able to produce the Ca:P levels we need to have of 1.0 or higher. Also notable was the fact that, although there were some significant differences seen within some of the species with regards to CP and fat, these differences were not likely biologically significant and were similar to the values seen by Baker *et al.* (1998). This shows that even large differences in protein and fat concentrations in the feeds do not greatly alter the protein and fat concentrations in the insects, thus choosing a gut-loading diet is not dependent on these two measures as a factor.

With this information, the second trial eliminated the OZTC and focused on the vitamin A differences in insects fed the control diet (PS) and the MBB and MHC diets. Previously measured vitamin A values in non-gut-loaded adult crickets and mealworms has been shown to be 0.8 IU/g, and superworms had measured values of about 0.95 IU/g (Baker *et al*., 1998). However, gut-loaded crickets have been shown to reach levels as high as 14.59 IU/g when measuring retinol and β-carotene (Li *et al.*, 2009). The level of accurate detection of vitamin A at the lab we sent our samples was 1.66 IU/g. Therefore, several of the samples tested showed below detectable levels. Surprisingly, the only cricket group to have detectable concentration of vitamin A was the PS diet (1.99 IU/g). There was no detectable vitamin A concentration in any of the superworm diets, and although the mealworms all had measurable levels of vitamin A (1.74-2.14 IU/g), there was no significant difference between the diets fed. When making our decision regarding which diet to choose for our protocols, this information removed vitamin A as a deciding factor.

Considering all of this information, we were left with Ca:P concentration and mortality as our two main deciding factors. The mortality with the MBB diet did not have a clear explanation. When the problem was first seen, samples of the diet were sent back to the manufacture to ensure that it met specification on nutrient composition and particle size. They confirmed that the product met all specifications. It is possible that the higher fat content of the diet could have caused a problem with the worms on a physical level. As the worms would crawl through the product, they could be getting coated with the oils, which may be making it harder for them to respire due to clogged trachea tubules. However, there may be ingredient items which could be affecting the worms in a different manner.

Conclusion

From the data, our current protocols were amended so that we will now go back to using the MHC gut-loader diet when gut-loading our crickets. We will continue to use the PS as our receiving and sustaining diet for non-gut-loaded insects, and we will be able to use both the PS and MHC as substrate for the mealworms and superworms, as there has never been a history of increased mortality on these diets. In the future, we will be exploring the use of different insect dusting products to help elevate Ca and vitamin A provided to our collection animals via our feeder insects.

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Table 1. Nutritional analysis of various feeder insect gut loading diets

Parameter	PS[1]	MHC[2]	MBB[3]	OZTC[4]
DM %	89.6	90.5	93.8	11.0
CP %	33.2	19.7	33.1	21.8
Crude Fat %	3.4	3.9	18.1	3.30
Ash %	7.64	25.70	26.67	11.84
Ca %	1.22	9.33	8.80	1.29
P %	0.89	0.58	0.89	0.64
Ca:P Ratio	1.37	16.09	9.89	2.02
Mg %	0.24	0.24	0.25	0.28
K %	1.23	0.90	0.72	3.27
Na %	0.17	0.21	0.38	0.36
Fe mg/kg	169	314	376	211
Zn mg/kg	104	79	83	60
Cu mg/kg	17	15	12	7
Mn mg/kg	87	76	75	55
Vitamin A IU/g	7.06	16.32	14.04	-

[1]Purina Game Bird + Turkey Startena – Control Diet
[2]Mazuri® Hi Calcium Gut Loading Diet #5M38
[3]Mazuri® Better Bug® Gut Loading Diet #5B45
[4]Oregon Zoo Tortoise Chop Diet (16% Bok Choy, 16% Broccoli, 12% Collard Greens, 19% Kale, 16% Shredded Carrot, and 21% Ground Mazuri® Tortoise LS Diet #5E5L; all on a DM Basis)

Table 2. Pre-trial mortality of mealworms (*Tenebrio molitor*) and superworms (*Zophobas morio*) fed Purina Game Bird + Turkey Startena or Mazuri® Better Bug® Gut Loading Diet as a bedding substrate or in a feeding dish for 48 hours.

Insect	**PSB[1]**	**MBBB[2]**	**MBBD[3]**
Mealworm Mortality %[4]	0.30	30.7	0.31
Superworm Mortality %[4]	0.00	100.00	0.83

[1]Purina Game Bird + Turkey Startena – Control Diet as Bedding Substrate
[2]Mazuri® Better Bug® Gut Loading Diet #5B45 as Bedding Substrate
[3]Mazuri® Better Bug® Gut Loading Diet #5B45 in Feeding Dish
[4]Mortality: # of dead insects at 48 hr/# of insects at 0 hr = % Mortality

Table 3. Nutritional analysis and mortality of crickets (*Acheta domesticus*) fed various gut loading diets over 48 hours.

Parameter	**PS[1]**	**MHC[2]**	**MBB[3]**	**OZTC[4]**	***P-values***
DM %	27.27[a]	27.37[a]	26.73[a]	24.90[b]	<0.001
CP %	60.63[ab]	59.90[b]	60.13[ab]	63.57[a]	0.04
Crude Fat %	24.47	26.30	25.83	25.30	0.29
Ash %	8.75[ab]	6.12[b]	6.89[b]	10.16[a]	<0.01
Ca %	0.17[c]	0.48[b]	0.88[a]	0.12[c]	<0.001
P %	0.86[b]	0.85[b]	0.90[a]	0.88[ab]	0.01
Ca:P Ratio	0.20[c]	0.57[b]	0.98[a]	0.13[c]	<0.001
Mg %	0.09[a]	0.09[a]	0.10[a]	0.08[b]	0.02
K %	1.17[ab]	1.15[b]	1.15[b]	1.24[a]	0.01
Na %	0.43[b]	0.42[b]	0.48[a]	0.50[a]	<0.001
Fe mg/kg	89.67[b]	81.67[b]	107.67[a]	66.00[c]	<0.001
Zn mg/kg	218.33[a]	203.00[b]	207.67[b]	225.67[a]	<0.001
Cu mg/kg	23.00	21.67	23.00	22.33	0.10
Mn mg/kg	45.00[a]	39.00[ab]	36.33[b]	34.67[b]	0.01
Mortality %[5]	5.60	5.73	7.73	6.13	0.40

[1]Purina Game Bird + Turkey Startena – Control Diet
[2]Mazuri® Hi Calcium Gut Loading Diet #5M38
[3]Mazuri® Better Bug® Gut Loading Diet #5B45
[4]Oregon Zoo Tortoise Chop Diet (16% Bok Choy, 16% Broccoli, 12% Collard Greens, 19% Kale, 16% Shredded Carrot, and 21% Ground Mazuri® Tortoise LS Diet #5E5L; all on a DM Basis)
[5]Mortality: wt of dead crickets at 48 hr/wt of crickets at 0 hr = % Mortality
[abc]Values with similar superscripts within the same row are similar ($P \leq 0.05$)

Table 4. Nutritional analysis and mortality of mealworms (*Tenebrio molitor*) fed various gut loading diets over 48 hours.

Parameter	PS[1]	MHC[2]	MBB[3]	OZTC[4]	*P-values*
DM %	41.37[a]	41.50[a]	40.00[b]	31.90[c]	<0.001
CP %	53.00[b]	53.57[b]	56.60[a]	56.33[a]	<0.001
Crude Fat %	32.87[a]	31.80[ab]	31.57[ab]	30.47[b]	0.03
Ash %	4.32	5.74	4.69	5.07	0.06
Ca %	0.15[c]	1.05[a]	0.55[b]	0.14[c]	<0.001
P %	0.79	0.80	0.79	0.78	0.09
Ca:P Ratio	0.19[c]	1.31[a]	0.70[b]	0.18[c]	<0.001
Mg %	0.22[a]	0.22[a]	0.20[b]	0.19[b]	<0.001
K %	1.01[a]	1.00[a]	0.94[b]	1.00[a]	0.02
Na %	0.15[ab]	0.13[b]	0.15[ab]	0.18[a]	<0.01
Fe mg/kg	79.00	86.33	75.33	67.00	0.10
Zn mg/kg	176.33	127.33	136.67	138.33	0.14
Cu mg/kg	23.00[a]	20.00[bc]	21.00[b]	19.33[c]	<0.001
Mn mg/kg	24.33[a]	18.67[b]	13.67[c]	13.67[c]	<0.001
Mortality %[5]	1.22	1.90	2.04	1.67	0.77

[1]Purina Game Bird + Turkey Startena – Control Diet

[2]Mazuri® Hi Calcium Gut Loading Diet #5M38

[3]Mazuri® Better Bug® Gut Loading Diet #5B45

[4]Oregon Zoo Tortoise Chop Diet (16% Bok Choy, 16% Broccoli, 12% Collard Greens, 19% Kale, 16% Shredded Carrot, and 21% Ground Mazuri® Tortoise LS Diet #5E5L; all on a DM Basis)

[5]Mortality: wt of dead mealworms at 48 hr/wt of mealworms at 0 hr = % Mortality

[abc]Values with similar superscripts within the same row are similar ($P \leq 0.05$)

Table 5. Nutritional analysis and mortality of superworms (*Zophobas morio*) fed various gut loading diets over 48 hours.

Parameter	PS[1]	MHC[2]	MBB[3]	OZTC[4]	*P-values*
DM %	42.03[b]	42.77[b]	47.37[a]	37.27[c]	<0.001
CP %	48.53	48.80	49.30	48.83	0.4
Crude Fat %	44.07[ab]	43.87[b]	45.67[a]	44.63[ab]	0.05
Ash %	6.95[a]	4.65[b]	3.76[b]	6.47[a]	<0.001
Ca %	0.06[b]	0.23[a]	0.33[a]	0.07[b]	<0.001
P %	0.66[a]	0.57[b]	0.58[b]	0.57[b]	<0.001
Ca:P Ratio	0.09[b]	0.41[a]	0.57[a]	0.13[b]	<0.001
Mg %	0.10	0.11	0.11	0.10	0.36
K %	0.65	0.70	0.66	0.67	0.12
Na %	0.09	0.12	0.10	0.11	0.08
Fe mg/kg	34.67[b]	39.00[ab]	44.33[a]	33.67[b]	<0.01
Zn mg/kg	77.00	76.33	76.67	75.67	0.79
Cu mg/kg	9.67	9.00	9.67	9.00	0.12
Mn mg/kg	8.67	9.33	9.00	8.00	0.15
Mortality %[5]	0.00[b]	0.00[b]	16.34[a]	0.00[b]	<0.001

[1]Purina Game Bird + Turkey Startena – Control Diet
[2]Mazuri® Hi Calcium Gut Loading Diet #5M38
[3]Mazuri® Better Bug® Gut Loading Diet #5B45
[4]Oregon Zoo Tortoise Chop Diet (16% Bok Choy, 16% Broccoli, 12% Collard Greens, 19% Kale, 16% Shredded Carrot, and 21% Ground Mazuri® Tortoise LS Diet #5E5L; all on a DM Basis)
[5]Mortality: wt of dead superworms at 48 hr/wt of superworms at 0 hr = % Mortality
[abc]Values with similar superscripts within the same row are similar ($P \leq 0.05$)

Table 6. Vitamin A[1] analysis of crickets (*Acheta domesticus*), mealworms (*Tenebrio molitor*), and superworms (*Zophobas morio*) fed various gut loading diets over 48 hours.

	Vitamin A (IU/g DM)			
Species	**PS[1]**	**MHC[2]**	**MBB[3]**	***P-values***
Cricket	1.99	ND[5]	ND[5]	-[6]
Mealworm	1.74	2.14	1.77	0.44
Superworm	ND[5]	ND[5]	ND[5]	-[6]

[1]Vitamin A assay measured as retinol and esters
[2]Purina Game Bird + Turkey Startena – Control Diet
[3]Mazuri® Hi Calcium Gut Loading Diet #5M38
[4]Mazuri® Better Bug® Gut Loading Diet #5B45
[5]Values that registered as present but below the level of detection (1.66 IU/g DM) are indicated as ND.
[6]Statistical analysis not done due to ND values.

CASE STUDY: WINOS FOR RHINOS: FEEDING GRAPE POMACE TO BLACK RHINOCEROS (*DICEROS BICORNIS*) AS A METHOD FOR MITIGATING IRON STORAGE DISEASE

Matthew A. Brooks, PhD*, Bob A. Lee, BA, and Jeffrey M. Pera, BS

Living Collections, Oregon Zoo, 4001 SW Canyon Rd., Portland, OR 97221, USA

Abstract

Iron storage disease has been detrimental to the captive browsing rhinoceros population. One of the proposed methods for reducing available iron in the gut of these animals is to increase iron chelators into the captive diet that are normally present in the wild diet. This study used the wine making by-product grape pomace, which is naturally high in tannins, as a browse item fed to two black rhinos over the course of two separate trials. The first trial, with the male rhino Ruka, showed promising results with increased fecal iron excretion and lowered serum iron levels when he was fed the grape pomace diet over a 10 day period compared to being fed bamboo leaves and stems as the browse item. However, serum indicators of iron storage status, ferritin, were increased. A second trial was conducted using both Ruka and his female cohort, Zuri. With the same design, serum iron levels did not respond as in the previous trial, but serum ferritin levels were again greater when the animals were fed grape pomace as the browse item. These results would indicate that the increased tannins in the diet did not improve iron storage status for these animals.

Introduction

Iron storage disease has been plaguing browsing rhinoceros species for decades (Candra *et al*., 2012; Olias *et al*., 2012). The species' inability to regulate the uptake of iron can be evidenced by the increased serum iron and transferrin saturation levels. More precise diagnosis of iron levels requires more invasive organ biopsies to determine tissue level iron. Because of the risk of such procedures, actual confirmation of iron storage disease in rhinos is generally not confirmed until post-mortem exams; however, these excess iron stores has been implicated in several clinical disorders observed in the species , such as hemolytic anemia, mucocutaneous ulcerative disorder, congenital leucoencephalomalacia, and decreased immune system response (Olias *et al*., 2012; Paglia and Tsu, 2012). Although work is still needed to help determine the exact genetic factors that relate to iron uptake in browsing rhinos, what is accepted is the regulatory mechanism that prevents excessive iron absorption most mammals, including grazing rhino species, has been lost over time in browsing rhinos species. This adaptation has evolved out of necessity for these animals that live in areas with low soil iron and subsist on plants high in iron chelators, such as tannins (Lavin, 2012). Although this adaptation serves the species well in the wild, captive managed rhinos are fed diets that are high in iron and low in chelators. The result is that most (75%) captive managed black rhinos will eventually succumb to the effects of this disease (Paglia and Tsu, 2012).

Recent work has suggested that captive diets could be improved for rhinos by adding back the chelators found in their natural diets through addition of either diet items naturally high in the compounds, oils and extracts rich in the compounds, or artificially produced chelators (Huntley, 2016, Lavin, 2012; Sullivan, 2016). Dietary chelators, such as naturally occurring tannins, bind strongly to iron in the digestive track. This bound iron would then pass through the rest of the tract

unabsorbed, effectively decreasing dietary iron as a method to mitigate the accumulation of iron in the rhinos. Red grape seeds and skins contain high levels of catechin, epicatechin, and gallic acid, which are three phytochemicals that will strongly bind to iron when given the opportunity (Yilmaz and Toledo, 2004). Through contacts within the Oregon Zoo Horticulture Department, a sizable amount of grape pomace was obtained from a regional winery. This pomace is the solid remains of the grapes after they have been pressed for their juice for making wine. This pomace contains the skins, pulp, seeds, and stems of the fruit. It was the intent of this study to evaluate the addition of this tannin-rich diet item to the diet of the Oregon Zoo black rhinos to determine its viability in mitigating dietary iron absorption.

Materials and Methods

Grape Pomace and Bamboo

The grape pomace used for this study was the by-product of the production of a rosé wine where the red grape skins and stems were pressed for their juice but not fermented with the wine as would be done with a red wine. The pomace was obtained from the Mt. Hood Winery and brought back to the Oregon Zoo where it was bagged into 2-gallon size freezer bags and stored at -20°C. Bamboo was collected on zoo grounds fresh daily, and stems and leaves were stripped from the stalk and bagged stored in a walk-in cooler kept at 2°C.

Feeding Trial A

During March of 2016, a feeding trial was conducted that included 10 days of our male rhino, Ruka, on a control diet (CON) with his normal diet and bamboo browse consisting of only leaves and stems, a 10 day wash-out period of his regular diet and no browse, and then a 10 day experimental diet with his normal diet and grape pomace (POM, Table 2). Both the POM and CON diets were designed to adjust the bamboo or grape pomace amounts to keep a consistent dry matter intake during the 10 day trial. During the trial, all feed was weighed in and all orts were weighed out to determine intake. The morning diet consisted of half of the hay diet. Browse was fed in the evening feeding with all of the grain and half of the hay portions of the diet. A blood sample was taken on each of the last three days of the CON and POM diet periods. During the last three days, total fecal collection was done to determine weight, and a subsample of feces was taken each day and composited over the three days. All fecal samples were stored in a -20°C freezer for later analyses.

Feeding Trial B

During March of 2017, a second feeding trial was conducted that included 10 days of both our male rhino, Ruka, and female rhino, Zuri, on a CON and POM diet with the same design as Trial A, outlined above (Table 2). The animals were fed all diets separately, but they were allowed access to each other in the yard when no diet was available. The only change between the protocols was to remove the fecal collection from the second trial.

Diet Analysis

All feed samples were collected, packed on dry ice, and sent to Dairy One Forage Laboratory (730 Warren Rd., Ithaca, NY 14850 USA) for proximate analyses. Feed samples and fecal samples were collected and sent on dry ice to the lab of Dr. Harley Naumann at the University of Missouri (110 Waters Hall, Columbia, MO 65211) for analysis of proanthocyanidins and protein precipitable phenolics. Proanthocyanidins were analyzed via acid butanol method (Hagerman, 2011) as

modified by Johnson (2014). Protein precipitable phenolics were analyzed by the method outlined by Hagerman and Butler (1978) as modified by Johnson (2014). These samples were then sent to the University of Missouri Agricultural Experimental Station Chemical Laboratories (700 Hitt Street, Columbia, MO 65211) for analysis of Fe, Zn, and Cu using AOAC Official Method 985.01(A, B, D).

Blood Analyses

Blood samples were always taken in the morning before the morning feed, can change over the course of a day, and fasting levels are preferred (Sauberlich, 1999). Blood was collected into serum separator tubs, centrifuged to separate serum, and serum was decanted into cryotubes and stored at -80°C before shipping. All blood samples were shipped to Kansas State Veterinary Diagnostic Laboratory (1800 Denison Ave., Manhattan, KS 66506) for analysis of serum iron, ceruloplasmin, ferritin, haptoglobin, and total iron binding capacity (TIBC). Transferrin saturation percentage was calculated as the serum iron/TIBC*100.

Results

Phenolic Analysis

Grape pomace was the only diet item to contain measurable values of proanthocyanidins and protein precipitable phenolics (Table 1). As all diet items were similar within each trial, except the browse component, only the mineral levels of the bamboo and grape pomace are shown in Table 1. Although Fe and Zn were greater in the bamboo than the grape pomace, both browse items contained physiologically low levels of all three minerals.

Fecal Output

Fecal output and mineral concentration was only recorded during Trial A of this study. During Trial A, intake, fecal output, and Fe, Cu, and Zn intake had comparable values between both diets (Table 3). Cu and Zn output were also comparable between the two diets tested. However, measured Fe output was higher than Fe intake for both diets, and Fe output was also numerically greater in the POM diet than in the CON diet.

Blood Analysis

As the *n* for this study was only 1 for Trial A and 2 for Trial B, no real statistical conclusions can be made from the data. What is observable from the data is that during both Trial A and B, serum iron did not seem to differ at a physiologically significant level, although it was slightly higher for Zuri during Trail B. Also, ceruloplasmin and TIBC remained consistent for both trials and treatments on both rhinos. Haptoglobin did not appear to differ between the CON and POM diets, but it was greater in Trail B compared to Trial A. Transferrin saturation percentage was lower in POM in Trial A, unchanged in Ruka in Trial B, and greater in POM with Zuri on Trial B. Ferritin values varied between Trial A and B, but was consistently seen was that ferritin levels were greater in the POM diets when compared to the controls within animal within trial.

Discussion

When this idea was initially introduced, a great deal of research went into the viability of this item as a feed source. Through some study into the field of oenology, it was determined that only the pomace from a certain wine would work for our situation. In the making of full bodied red wines, such as a Cabernet Sauvignon or Burgundy, the grapes and skins and stems are pressed and then

fermented with the wine. The resulting grape pomace, therefore, contains a high alcohol and lower sugar content than would be suitable for feeding to rhinoceros. White wines do not ferment the skins and stems with the wine, but they use only white grapes. This resulting pomace would be too low in tannins for the purposes of our study. However, when a lighter bodied red wine is made, such as a Pinot Noir or Rosé, the amount of time the grape skins come in contact with the juice is limited to a short period, typically 4 hr to 2 days. This does not allow for enough time for the sugars to be converted to alcohol in large amounts, resulting in a grape pomace that is high in tannins and sugars and low in alcohol. Nutritionally, grape pomace is a great browse feed that is 42% DM, 12% CP, 28% ADF, and only 80.5 ppm Fe. The added practical benefits to the use of grape pomace were that it is a waste product for the producer that they will gladly give away for free, it can be easily bagged into useable amounts with very little effort, and it can be stored in a freezer and thawed readily to be fed out to the collection animals. Nutritionally, the grape pomace is very similar to the control browse we chose, bamboo. Bamboo leaves and stems are 52% DM, 13% CP, 29% ADF, and only 165.5ppm Fe. Further, the grape pomace had a greater palatability, which led to increased consumption of overall diet by our female rhino, Zuri, who is normally a very reluctant eater.

Initial results from Trial A showed promising results with increased iron excretion and possibly decreasing serum iron when Ruka was fed the POM diet. Also, the decreased serum iron levels and stable TIBC levels indicated decreased transferrin saturation percentage when Ruka was fed POM. However, fecal results were showing that there were endogenous iron sources in the fecals that were unable to be accounted for and were making fecal iron excretion greater than fecal intake. What was seen was an increase in ferritin on the POM diet. As ferritin is the serum indicator for iron stores, this lead us to question if this method would lead to a viable high tannin feed source. Previous work has shown that there can be individual variation regarding serum parameters and effectiveness of chelators (Sullivan *et al.*, 2015), so a second trial was proposed to determine if the results could be repeated in the same rhino and also in his female cohort.

Trial B included both of our Black rhinos, Ruka and Zuri. Both animals are young rhinos, 6 yrs and 9 yrs old, respectively. From 2015 until 2017, their normal ferritin levels range between 480-2200 ng/mL, as measured monthly, with their average values being 887 ng/mL for Zuri and 1413 ng/mL for Ruka. Two dietary changes occurred between the first and second trail. First, the rhinos were both switched off of the higher iron/protein alfalfa hay in favor timothy had and more orchard grass hay. Second, produce was increased in the diet to accommodate shifting and training. However, since Ruka is still growing, his overall intake was increased, which lead to an increased overall iron intake. Fecal collection was done as part of Trial B due to the labor required and the confounding factor of the endogenous iron seen in the first trial. Serum parameters in Ruka showed similar numbers in both Trials, with the exception of haptoglobin being increased and ferritin values decreasing in both the CON and POM diets. What did appear to remain consistent through all three trials was that when fed the POM diet, ferritin levels were between 20-70% greater compared to the CON diet.

Ferritin can be affected by many factors including time of day, age, sex, fasting or fed state, or inflammation (Sauberlich, 1999). Ceruloplasmin and haptoglobin were measured in the serum as indicators of inflammation. As values remained consistent within each rhino within each trial, there was no indication that inflammation was causing the increase in serum ferritin. Further, these

animals were kept on the POM diet for another 14 days after Trial B was concluded without any breaks. The ferritin value obtained from Zuri on day 23 of being the POM diet was 1126 ng/mL with the other values remaining consistent to what was seen during the trial, which indicated a sustained increased ferritin iron load compared to her normal diet.

Conclusion

What was concluded from this study, was the initial promising results seen when feeding grape pomace browse as a natural chelator of iron in the diet did not overcome the negative results seen in the serum ferritin levels. The elevated serum ferritin levels measured during each portion of the trials where the animals were fed grape pomace as a browse item indicate that the use of the grape pomace is increasing the iron load on the animals, or, it is at least not improving the iron status beyond just feeding a low iron diet.

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Table 1. Mineral and phenolic concentration of bamboo leaves and stems and grape pomace fed as browse to black rhinoceros (*Diceros bicornis*) to evaluate the effects on iron storage disease.

Parameter	Unit	Bamboo Leaves and Stems	Grape Pomace
DM	%	52	42
Fe	ppm DM	165.5	80.5
Zn	ppm DM	86.0	14.3
Cu	ppm DM	10.2	13.6
Proanthocyanidins	g/kg DM	UD[1]	45.9
Protein Precipitable Phenolics	g/kg DM	UD[1]	22.2

[1]Undetectable levels

Table 2. Nutritional analyses of diets fed to two black rhinoceros (*Diceros bicornis*) to evaluate the effect of grape pomace on iron storage disease.

		Ruka Trial A[1]		Ruka Trial B[2]		Zuri Trial B[2]	
Diet Item	**Unit**	**Control**	**Pomace**	**Control**	**Pomace**	**Control**	**Pomace**
Apple	g/d	220		1760		1760	
Carrot	g/d	-		500		500	
Emcelle Tocopherol	g/d	13		13		13	
Mazuri ZNN Herbivore Diet #5M64	g/d	2000		2400		4200	
Mazuri Browser Rhino Cube #5Z1P	g/d	1000		1200		1200	
Alfalfa Hay	g/d	5000		-		-	
Orchard Grass Hay	g/d	5000		6000		8000	
Timothy Hay	g/d	-		6000		6000	
Bamboo Leaves and Stems	g/d	1800		1800		2200	
Grape Pomace	g/d		2400		2380		2910
Parameter							
DM	%	81.4	80.9	78.0	75.7	79.6	77.3
CP[3]	%	15.4	14.2	11.6	11.5	12.2	12.0
Crude Fat[3]	%	2.7	2.5	2.7	2.7	2.8	2.8
NDF[3]	%	47.0	49.5	57.2	55.0	55.8	53.7
ADF[3]	%	31.3	32.2	32.8	32.7	32.0	31.8
Ash[3]	%	7.6	7.8	6.8	6.7	7.0	6.8
DM Intake	kg/d	12.64		15.34		19.00	
Iron	mg/d	1749	1798	1883	1932	2373	2433
Copper	mg/d	246	240	239	233	318	310
Zinc	mg/d	742	721	877	855	1196	1170
Protein Precipitable Phenolics[4]	g/d	0	20.9	0	20.8	0	25.4

[1]Trail A was conducted March 2016

[2]Trial B was conducted March 2017

[3]DM Basis

Table 3. Mineral intake and output over a 3-d period[1] of a black rhinoceros (*Diceros bicornis*) fed for 10 days on a diet which included either bamboo browse or grape pomace browse.

Parameter	Unit	Ruka Trial A	
		Control Diet[2]	Grape Pomace Diet[3]
Intake	kg DM/3 d	38.1	38.2
Fecal Output	kg DM/3 d	25.8	24.0
Protein Precipitable Phenolics	g/3 d	0.0	62.9
Fe Intake	mg/3 d	13368	12422
Fe Output	mg/3 d	14835	17904
Cu Intake	mg/3 d	506	551
Cu Output	mg/3 d	387	415
ZN Intake	mg/3 d	2523	1672
ZN Output	mg/3 d	1780	1752

[1]Each diet was fed for 10 days with a 10-day wash-out period in between each feeding. All parameters were measured over the last 3 days of each 10 day feeding period.

[2]Diet consisted of 33% Alfalfa hay, 33% Orchard Grass hay, 13.4% Mazuri ZNN Herbivore Diet #5M64, 7% Mazuri Browser Rhino Cube #5Z1P, 1.5% Apple, 0.1% Emcelle® Tocopherol, and 12% Grape Pomace

[3]Diet consisted of 32% Alfalfa hay, 32% Orchard Grass hay, 13% Mazuri ZNN Herbivore Diet #5M64, 6% Mazuri Browser Rhino Cube #5Z1P, 1.5% Apple, 0.1% Emcelle® Tocopherol, and 15.4% Grape Pomace

Table 4. Serum parameters of two black rhinoceros (*Diceros bicornis*) fed for 10 days on a diet which included either bamboo browse or grape pomace browse.

		Ruka Trial A[1]		Ruka Trial B[2]		Zuri Trial B[2]	
Serum Parameter[3]	**Unit**	**Control**	**Pomace**	**Control**	**Pomace**	**Control**	**Pomace**
Iron	µg/dL	192.67	163.00	176.33	182.00	171.67	245.33
Ceruloplasmin	mg/dL	113.33	87.00	89.60	81.83	87.37	91.10
Ferritin	ng/mL	1457.33	2035.33	849.67	1450.67	830.33	1028.33
Haptoglobin	mg/dL	118.00	108.67	702.00	607.67	567.00	583.00
TIBC[4]	µg/dL	320.00	316.33	339.00	353.67	406.67	383.33
Transferrin Saturation[5]	%	60.00	52.00	52.02	51.52	42.32	63.99

[1]Trial A was conducted March 2016, and daily diets included Alfalfa hay, Orchard Grass hay, Mazuri ZNN Herbivore Diet #5M64, Mazuri Browser Rhino Cube #5Z1P, Apple, Emcelle® Tocopherol, and either Bamboo leaves and stems (Control) or Grape Pomace (Pomace). Iron content of both diets was 140 ppm.

[2]Trial B was conducted March 2017 and daily diets included Timothy hay, Orchard Grass hay, Mazuri ZNN Herbivore Diet #5M64, Mazuri Browser Rhino Cube #5Z1P, Apple, Emcelle® Tocopherol, and either Bamboo leaves and stems (Control) or Grape Pomace (Pomace). Iron content of both diets was 125 ppm.

[3]Each diet as fed for 10 days with a 10-day wash-out period between the Control and Pomace diets. All serum parameters are the average of 3 blood samples taken on the last three days of each 10-day portion of the trials.

[4]Total iron binding capacity

[5]Transferrin Saturation = Iron/TIBC*100

BASELINE INTAKE STUDY FOR FOUR SPECIES OF PRIMATES IN CAPTIVITY: *CALLITHRIX PYGMAEA*, *NYCTICEBUS PYGMAEUS*, *PROPITHECUS COQUERELI* AND *CALLITHRIX GEOFFROYI*

Timothy J. Brunner[1]* and Barbara Toddes BS, GC[2]

[1]Purdue University, College of Agriculture, 615 West State Street, West Lafayette, IN 49707-2053
[2]Philadelphia Zoological Garden, Nutrition Department, 3400 West Girard Avenue, Philadelphia, PA 19104-1196

Abstract
Little information is known in regards to the natural or seasonal diets of primates. In captivity, nutrition can be an important part of preventative medicine and lead to an improved quality of life for primates. To better understand the nutrient intake of four primate species in the collection at the Philadelphia Zoo, we conducted an intake study over a minimum of four days for each species. A spreadsheet was used to track the amounts consumed and convert these numbers to nutrient values. Furthermore, we attempted to correlate known health issues to the nutrients consumed. Finally, we compared diet appropriateness to the gut morphology of each species, where data was available. Our results show an accepted sugar to fiber ratio of 3.29:1, 6.46:1, 10.16:1, and 0.78:1 for the pygmy loris (*Nycticebus pygmaeus*), Geoffroy's marmoset (*Callithrix geoffroyi*), pygmy marmoset (*Callithrix pygmaea*), and Coquerel's sifaka (*Propithecus coquereli*), respectively. Our goal was to establish an appropriate, subjective sugar to fiber ratio for each primate. Although there are possible correlations between health issues and consumed nutrients, these were not confirmed and need further evaluation. Overall, this information will be used to better the diets of the primates in the collection at the Philadelphia Zoo.

ANALYSIS OF FATTY ACID PROFILES IN EASTERN BOX (*TERRAPENE CAROLINA CAROLINA)* AND COMMON SNAPPING *(CHELYDRA SERPENTINE)* TURTLES FOR WILD AND IN-HUMAN CARE ENVIRONMENTS

Khushboo Dass, BS[1], Kimberly Ange-van Heugten, PhD[1]*, Elizabeth A. Koutsos, PhD[1,2]*, and Larry J. Minter, MS, DVM, Dipl. ACZM[3,4]

[1]Department of Animal Science, North Carolina State University, Raleigh, NC 27695, USA
[2]Koutsos Consulting LLC, 2084 Toad Hollow Trail, Apex, NC 27502, USA
[3]North Carolina Zoo, 4401 Zoo Pkwy, Asheboro, NC 27205, USA
[4]Department of Clinical Sciences, North Carolina State School of Veterinary Medicine, Raleigh, NC 27606, USA

Abstract

Diets of wild animals are often more diverse and offer higher concentrations of nutrients than those of animals' in human care (zoos, rehabilitation facilities, etc.). Managing wild animals within human care facilities is often necessary, and we hypothesized that chelonian dietary differences within circulating fatty acid profiles would be reflected in wild *vs.* human care data. The current study examined the effect of species and environment on fatty acids concentrations in two omnivorous species of chelonians native to North Carolina within two environments: Eastern box turtles (*Terrapene carolina carolina)* and common snapping turtles (*Chelydra serpentine*) located in the wild and in-human care. Whole blood was collected and placed on spot cards for later analysis of all 26 fatty acids in a total lipid fatty acid profile. This novel research indicated that snapping turtles have significantly ($P < 0.05$) higher values of Linolenic acid, Dihomo-γ-Linolenic acid (DGLA), Tetradonic Acid, Docasatetraenoic acid (DTA), Docosapentaenoic acid (DPA), Eicosadienoic acid, Erucic acid, and overall saturated fatty acids. Among all the wild animals, there tended ($P < 0.06$) to be higher values for α-Linolenic acid, DGLA, Arachidonic acid, Eicosadienoic acid, Eicosatrienoic acid, DPA, and DTA. Docasonic acid, DTA, DPA, Eicosadienoic acid, and Nervonic acid showed significant ($P < 0.01$) differences via species x environment interactions. Interestingly, both wild species showed higher concentrations of Dihomo-γ-Linolenic acid (20:3n6), known to be directly affected by diet and display anti-inflammatory effects. This research may allow us to better formulate diets for chelonian kept in human care. Additionally, fatty acids are used for many important body functions including proper immune system usage and therefore our research provides new biologically important data for the reptile diagnostic field.

USING STRAW NOTES TO STANDARDIZE AND IMPROVE ZOO NUTRITION OUTCOMES

Andrea L. Fidgett, PhD, Katherine. R. Kerr, PhD, Michele Gaffney, MS*

San Diego Zoo Global, San Diego, CA 92112, USA

Abstract

Nutritional inquiries are widely diverse in topic, background detail, and background of our intended audience and inquirers. Providing the required information in an organized and standardized framework can expedite the progression and/or resolution of the inquiry. Developed by nutritionists at San Diego Zoo Global, the STRAW note – an mnemonic acronym for **S**ituation, **T**arget, **R**equired **A**ction(s) by **W**ho/**W**hen – is an easy-to-remember framework for documenting zoo nutrition questions, particularly those involving multiple stakeholders across departments

Situation: San Diego Zoo Global (SDZG) is a not-for-profit organization that operates the San Diego Zoo, the San Diego Zoo Safari Park, and the Institute for Conservation Research. Combined, the Zoo and Safari Park are home to more than 8,000 animals representing more than 800 species. Nutritionists serve both collections and field questions on a daily basis concerning feeding, diet, and nutrition from animal care staff, veterinarians, vet technicians, pathologists, and the browse team (a subset of the Horticulture Department). Because the stakeholders come from a range of backgrounds and experience, questions raised differ substantially in background detail provided, typically resulting in follow-up correspondence to clarify the request before progressing the inquiry. Example zoo nutrition questions include: "Do you know if watermelon is ok for aye-aye?" "Is crepe myrtle a potential browse species?" "Can we get a diet increase for … (insert species/specimen)"

Target: Develop a standardized method for recording and responding to questions in note form. The note format must be sufficiently detailed to ensure someone with no previous interaction can obtain all the necessary information from the record and/or subsequent consultation to appropriately provide care/follow-up. Efficiency and time management are also important (particularly in clinical nutrition cases), so the note must also avoid overly wordy phrasing and unnecessary detail. Headings for the note must be easy to follow, sufficiently broad to fit the variety of questions received, and outcome driven.

Required Actions: Review alternative documentation note formats. SMART is a mnemonic acronym giving criteria that guides in the setting of objectives, for example, in project management, employee-performance, and personal development. Each letter in SMART refers to a different criterion for judging objectives, which are easy to understand. The SOAP note – a mnemonic acronym for **S**ubjective, **O**bjective, **A**ssessment, and **P**lan – is a documentation format used among the veterinary profession (including SDZG Veterinary Services) to record patient interactions. Neither SMART nor SOAP readily apply to questions about feeding, diet, and nutrition, nonetheless their definition informed STRAW.

When: Using a standardized recording format to respond to nutrition inquiries, all staff can approach any diet or nutrition-related question and work towards a positive outcome in a logical manner. The STRAW note can stand on its own, providing a comprehensive review of the case, and the thought processes at each step should be evident to anyone reviewing the record. Use of the STRAW note format enhances communication between all staff across departments, optimizing the quality of care and minimizing the potential for redundancy and mistakes. It serves as a record of the question, agreed targets, communications with stakeholders, and reasoning for any action (or lack of action). STRAW notes can be shared in various formats suitable for archiving and future retrieval (*e.g.* email and Zoo Information Management Systems)

Situation	Details of inquiry and why it was prompted Brief summary of key points from stakeholder discussions, including subjective information Available objective information Appropriate historical information
Target	Target as determined by discussions with stakeholders Target may be a set goal or an evaluation of inquiry itself (i.e., feasibility, need for revision, etc.)
Required **A**ction(s)	Key steps to achieve desired outcome a.k.a. what will happen?
Who/**W**hen	Who is responsible for the actions (including follow-up), by when?

HAND-REARING A HIPPOPOTAMUS (*HIPPOPOTAMUS AMPHIBIUS*): A RARE OPPORTUNITY FOR COLLABORATION

Barbara Henry, MS[1]*, Michael L. Power, PhD[2], and Michael T. Maslanka, MS[3]

[1]Cincinnati Zoo & Botanical Garden, Cincinnati, OH, 45220, USA
[2]Nutrition Laboratory and Conservation Ecology Center, Smithsonian Conservation Biology Institute, National Zoological Park, 3001 Connecticut Ave NW, Washington DC 20008, USA
[3]Smithsonian Conservation Biology Institute, National Zoological Park, Center for Animal Care Sciences, Department of Nutrition Science, 3001 Connecticut Ave., Washington, DC 20008, USA

Abstract
On January 24, 2017, Cincinnati Zoo & Botanical Garden had a premature hippopotamus (*Hippopotamus amphibious*) calf (Fiona) born to a first-time mom (Bibi) on land weighing 13.4 kgs. After one and a half hours, Fiona was pulled due to no attempts to stand or nurse coupled with lack of maternal care; Fiona was cold to touch and needed supportive care. There were no recorded documents of hippo hand-rearing in the US to date. Gray (1960) published one sample of nutritional parameters from a hippo milked at eleven weeks of lactation. Using that as a base, a formula was devised and offered to Fiona after one day of offering Pedialyte®. Due to previous training, Bibi allowed milk collection. Most of the expressed milk was offered to Fiona; a small sample was collected for analyses. Milk collection continued through the first two weeks of lactation when Bibi appeared to end lactation, providing four subsamples for nutrient analyses. Samples were sent to the Milk Repository in the Department of Nutrition Science at the Smithsonian National Zoological Park and Conservation Biology Institute. Using the provided nutrient analyses, the formula was adjusted to more closely mimic dam's milk. The formula contained: water, Zoologic® milk matrix 33/40 and 42/25 (Pet Ag, 255 Keyes Ave., Hampshire, IL 60140) with a small percent of a 50% dextrose solution. Due to premature birth and digestive issues the formula was offered at a diluted rate and increased slowly ensuring consistent weight gain. The final formula for digestion and growth was reached after three months of increasing concentration.

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TALES FROM AN AQUATIC NUTRITIONIST: APPLYING FIELD STUDIES ON ELASMOBRANCHS TO MANAGED COLLECTIONS

Lisa A. Hoopes, PhD

Department of Research and Conservation, Georgia Aquarium, 225 Baker Street NW, Atlanta, GA 30313, USA

Abstract

Proper nutrition is the cornerstone of good health and a key component of preventative medicine programs at zoos and aquariums. Nutritional studies at zoological facilities have largely focused on terrestrial species due to relative ease in handling, feeding, and sampling, compared to their aquatic counterparts. Despite a growing number of elasmobranch species maintained in zoos and aquaria, little is known about species-specific nutrient requirements, and literature on wild diets are often lacking. Field studies on elasmobranchs provide a unique opportunity to collect non-invasive samples to understand baseline health and nutritional data on wild populations, which can be used to gauge the management of dietary health in aquarium collections. Blood sampling can provide information on trace mineral, vitamin, fatty acid, and heavy metal levels; while tissue sampling can provide information on trophic ecology and potentially, individual prey items in the diet. Ongoing field studies with spotted eagle rays (*Aetobatus narinari*), southern stingrays (*Dasyatis americana*), and sand tiger sharks (*Carcharias taurus*) suggest that there is room for improvement in managing the dietary health of these species. Additionally, the development of baseline data from wild populations may provide guidelines to assess not only health, but reproductive status, in managed elasmobranch collections.

BROWSER NUTRITION – PAST, PRESENT AND FUTURE

Jürgen Hummel, PhD[1]*, Isabel Gussek, PhD[2,3], and Marcus Clauss, PhD, MSc, Dipl. ECVC[4]

[1]Department of Animal Sciences, University of Goettingen, Kellnerweg 6, 37077 Göttingen, Germany
[2]University of Bonn, Institute of Animal Science, Endenicher Allee 15, 53115 Bonn, Germany
[3]Federal Office for Agriculture and Food, Deichmanns Aue 29, 53179 Bonn, Germany
[4]Clinic for Zoo Animals, Exotic Pets and Wildlife, University of Zurich, Winterthurerstrasse 260, CH-8057 Zurich, Switzerland

Abstract
During the last decades, browser nutrition has received constant attention. Besides feeding preferences/feeding type, their body size should be considered when designing appropriate diets; potential feeds can be ranked according to their chemical (e.g. fermentative behavior) and mechanical properties (e.g. abrasiveness).

Large browsers like giraffe, moose, or okapi have been identified as particularly challenging to feed. Recent studies indicate the quality of their diets in the wild to be not as high as sometimes assumed (Steuer *et al*. 2014), while being of low abrasiveness (Hummel *et al*. 2011) - both facts proving concepts of essentials of their feeding in captivity.

A recent study on practical giraffe nutrition in German zoos gives indication for negative effects of e.g. excessive concentrate/produce feeding (Gussek *et al*., in press). This study also quantified the differences in fermentation rates (Gussek *et al*. 2016).

In the future, projects on appropriate protein levels in large browser diets appear on the agenda; on the side of basic research, phylogenetic influences (bovids vs. giraffids/cervids) on dietary adaptability of browsers appear worth investigation.

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EVALUATION OF DIET INFLUENCE ON OXIDATIVE STRESS AND ITS IMPACT ON SEMEN QUALITY IN SNOW LEOPARDS (*UNCIA UNCIA*)

Cayla J. Iske, MS[1]*, Cheryl L. Morris, PhD[1,2], Jason R. Herrick, PhD[2]

[1]Animal Science Department, Iowa State University, 806 Stange Rd, Ames, IA 50011, USA
[2]Omaha's Henry Doorly Zoo & Aquarium, 3701 S 10th St, Omaha, NE 68107, USA

Abstract

The snow leopard (*Uncia uncia*) population in zoos has been declining over the past two decades, and since 2008, less than 30% of recommended breeding pairs have produced litters. Male fertility is a possible factor influencing reproductive success, which may be influenced by diet and oxidative stress (OS). The objective of this study was to evaluate dietary influence on OS markers and the relationship between these parameters and ejaculate traits in snow leopards (n = 14) maintained in U.S. zoos (n = 12). Diet samples were taken and cats were immobilized during the breeding season (January to June) for reproductive exams and blood collections. Samples were analyzed for chemical composition (diet and blood), antioxidant capacity (blood and seminal fluid), and markers of OS (blood and seminal fluid), and correlations between these variables and reproductive traits (total number of sperm per ejaculate and the proportion of spermatozoa with normal morphology) were analyzed by linear regression. Antioxidant enzymes (superoxide dismutase [SOD] and glutathione peroxidase [GPx]) and ferric reducing antioxidant potential (FRAP) are protective against OS, while protein carbonyls (PC), thiobarbituric acid reactive substances (TBARS), and DNA/RNA damage are indicative of OS. Retinol acetate and phosphorus intakes were positively correlated ($P < 0.05$) with antioxidant enzymes in blood (SOD, $R^2 = 0.32$; GPx, $R^2 = 0.56$, respectively), while iron intake was positively correlated ($P < 0.05$) with markers of OS damage in blood (PC, $R^2 = 0.47$; TBARS, $R^2 = 0.33$). In seminal fluid, copper and α-tocopherol intakes were negatively correlated ($P < 0.05$) with SOD ($R^2 = 0.76$) and FRAP ($R^2 = 0.73$). Total sperm count was positively correlated ($P < 0.05$) with lauric acid intake ($R^2 = 0.72$). The proportion of spermatozoa with normal morphology was positively correlated ($P < 0.05$) with dietary copper intake ($R^2 = 0.63$) and negatively correlated ($P < 0.05$) with SOD activity in seminal fluid ($R^2 = 0.90$). Retinol acetate and phosphorus may reduce OS by increasing blood antioxidant enzyme activity. Dietary copper and α-tocopherol, appear to negatively impact seminal fluid antioxidant enzymes; however, dietary α-tocopherol improved sperm morphology. Vitamin A increased blood antioxidant enzyme activity and sperm count but reduced normal sperm morphology. Therefore, the relationship between blood and seminal fluid markers and influence of diet warrants further investigation. In conclusion, fatty acid, mineral, and vitamin A, and E intakes may influence OS and sperm quality. These results suggest that altering the nutrient content of diets may influence OS and potentially improve ejaculate quality in male snow leopards.

REGURGITATION AND REINGESTION IN CAPTIVE GREAT APES

Kelly Kappen, MS[1]*, Cayla Iske, MS[2], Roni McClellen, BS[1], and Cheryl Morris, PhD[1]

[1]Omaha's Henry Doorly Zoo & Aquarium, 3701 S 10th St, Omaha, NE 68107, USA
[2]Animal Science Department, Iowa State University, 806 Stange Rd, Ames, IA 50011, USA

Two male western lowland gorillas (*Gorilla gorilla gorilla*) and one female northwest Bornean orangutan (*Pongo pygmaeus pygmaeus*) at Omaha's Henry Doorly Zoo and Aquarium have exhibited recurring regurgitation and reingestion (R&R) behavior. No negative health effects have been noted related to this behavior in the two gorillas; however, the orangutan has had multiple cavities that are suspected to be related to R&R. In addition to the potential health ramifications, R&R is considered an undesirable behavior for zoo visitors, and though it is common in captive apes, it has not been observed in wild populations. Our goal was to reduce or eliminate R&R behavior in the two affected gorillas by implementing a biscuit-free/low-starch diet suggested by several other zoos. In the two gorillas, this treatment had the unfortunate effect of increasing R&R behavior to more than double its previous frequency. Due to the lack of success in reducing R&R behavior in these gorillas with the implementation of the biscuit-free/low-starch diet, and once again upon the recommendation of other zoos, our next strategy was to try providing daily access to browse for affected individuals. Daily browse was offered to one gorilla and one orangutan. Initial results seem promising but inconsistent, and continued observations are needed.

Points to discuss:

- Have others had similar issues in great apes? Have these been resolved or are they ongoing?
- Have other animals who exhibit consistent R&R had any related medical issues or is it considered strictly a behavioral problem?
- What metrics have been used to determine success?
- Are there factors other than diet that might affect this behavior?

CAROTENOID GUT-LOADING OF CRICKETS (*ACHETA DOMESTICA*) AND MEALWORMS (*TENEBRIO MOLITOR*)

Erin Kendrick, MS* and Michael T. Maslanka, MS

Department of Nutrition Sciences, Smithsonian Conservation and Biology Institute, National Zoological Park, 3001 Connecticut Ave NW, Washington, DC 20008, USA

Abstract

The lack of diversity of invertebrate prey items available to feed captive animals limits available nutrients, requiring manipulation of the diet fed to these prey items prior to offering to other animals. Of late, much of the gut-loading research and recommendations focused on correcting the Ca:P imbalance inherent to the insects available through commercial production. More recently, attention has shifted to also find ways to improve levels of other nutrients, including carotenoids. Increasing carotenoids, in particular, may allow for maintaining appropriate coloration of species reliant on those cues for breeding and impact breeding success, health, and immune function. We will discuss the results of using several limited-ingredient mixes (1-3 ingredients) for gut-loading mealworms (*Tenebrio molitor*) and multiple sizes of crickets (*Acheta domestica*), under typical captive conditions. The gut-load recipes were intended to increase carotenoid levels while maintaining appropriate balance of other nutrients, specifically calcium and phosphorus.

IMPLICATIONS OF MANAGING A MOTHER-REARED BORNEAN ORANGUTAN (*PONGO PYGMAEUS*) INDOORS DURING EARLY INFANCY

Erin Kendrick, MS* and Michael T. Maslanka, MS

Department of Nutrition Sciences, Smithsonian Conservation and Biology Institute, National Zoological Park, 3001 Connecticut Ave NW, Washington, DC 20008, USA

In September, 2016 a healthy, male Bornean orangutan (*Pongo pygmaeus*) was born and raised by the dam at the National Zoological Park. However, due to enclosure limitations and timing of the year, the pair would have no access to the outdoor enclosure until May. Knowing that milk was not likely to contain biologically significant levels of vitamin D, and access to UV lighting was not practical, we were faced with the potential of rickets developing in the infant without supplementation. This discussion will revolve around the science behind practical vitamin D provision to a neonate primate in a northern temperate climate.

Points to discuss:

- Would we expect vitamin D_3 stores would last until the spring?
- Do we think (or know) orangutan milk would supply adequate vitamin D?
- What are our options for supplementation?
- If we do supplement, can we test efficacy?

GRIZZLY BEAR (*URSUS ARCTOS)* DIET MANAGEMENT: SEASONAL DIETS TO ATTAIN HEALTHY WEIGHT

Katherine Kerr, PhD[1]*, Hali O'Connor, BA[2], Jacob Shanks, BS[2], Gaylene Thomas[2], and Chris Hamlin, BS[2]

[1]San Diego Zoo Global, San Diego, CA 92112, USA
[2]San Diego Zoo, San Diego, CA 92112, USA

Abstract

Grizzly bears (*Ursus Arctos*) are specially adapted to survive the changing seasons of northern latitudes through changes in metabolism and behavior. In the zoo environment, these adaptations make weight management a challenge (Watts, 2009). Wild bears pack on the pounds during warm months to ensure they can survive the winter's harsh weather and scarcity of food - losing as much as one-third of their body weight during hibernation (Robbins *et al*., 2005; Kingsley *et al*., 1983). At San Diego Zoo, the grizzly bears still pack on the pounds in warm months, but their winter isn't so harsh. In the past, food has been plentiful, resulting in bears that were overweight all year round. In January 2016, Nutritional Services and Animal Care Staff at San Diego Zoo partnered to develop a year-long diet plan that included seasonal shifts in when food energy is offered to bears and selection of dietary items with lower caloric density. Our goal was to encourage more appropriate seasonal fluctuations in body condition and body weight. Given their unique biology, success of the program could not be assessed until 1 year later: Through caloric restriction in the winter and spring of 2016 and 2017, we were able to attain seasonal changes in body weight of approximately 40 kg (15% of BW; double previous years). Efforts have continued in 2017 with a refined process and hopes for further success in weight management tailored to these seasonal animals.

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Table 1. Composition of diet (% As-Fed) offered to grizzly bears (*Ursus arctos*) at San Diego Zoo in 2015.

Item[1]	Unit	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Extruded Dry Diet	%					1	5	5	5	5	5	2	
Raw Meat Items	%	15	18	33	36	36	37	38	38	37	37	37	20
Whole Prey	%	6	6	5	4	4	6	5	5	7	7	5	5
Produce	%	78	76	62	60	58	52	53	53	51	51	56	75

[1]Extruded Dry Diet = Omnivore Diet (Mazuri® #5635); Raw Meat Items = commercial raw meat diets, muscle meats, organ meats, and meat on bone; Whole Prey = whole rabbit and fish; Produce = greens, vegetables and fruit

Table 2. Composition of diet (% As-Fed) offered to grizzly bears (*Ursus arctos*) at San Diego Zoo in 2016.

Item[1]	Unit	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Extruded Dry Diet	%						2	3	4	8	6		
Raw Meat Items	%	20	19	20	21	24	25	25	25	27	30	34	21
Whole Prey	%				3	8	7	7	7	6	9	7	4
Produce	%	80	81	80	75	67	64	63	62	58	53	57	73
Cellulose	%				1	2	2	2	2	2	1	2	1

[1]Extruded Dry Diet = Omnivore Diet (Mazuri® #5635); Raw Meat Items = commercial raw meat diets, muscle meats, organ meats, and meat on bone; Whole Prey = whole rabbit and fish; Produce = greens, vegetables and fruit

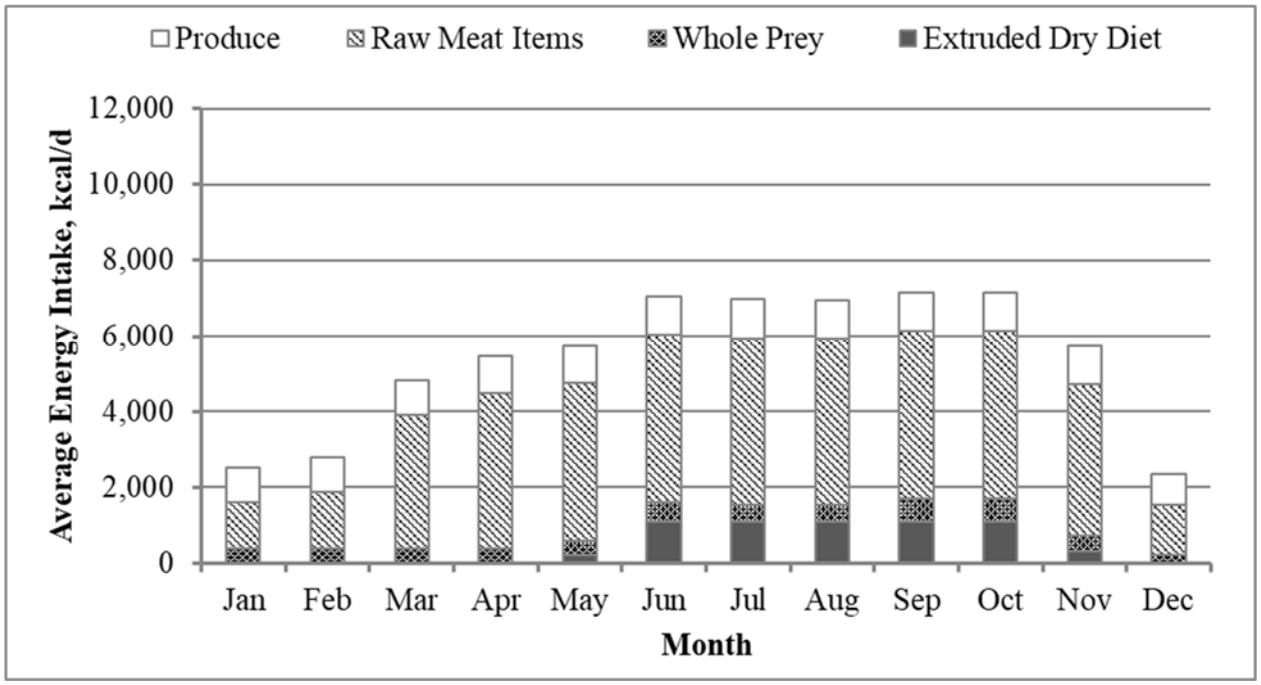

Figure 1. Average energy intake (kcal/d) of male grizzly bears (*Ursus arctos*) housed at San Diego Zoo in 2015.

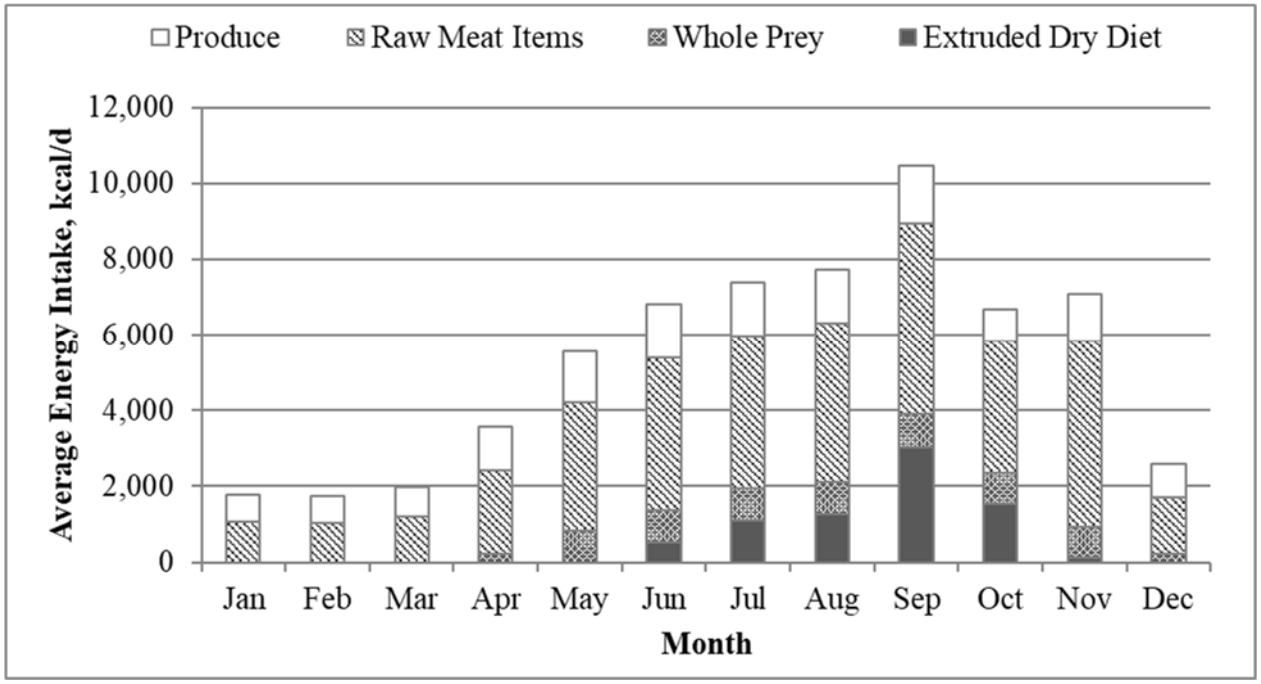

Figure 2. Average energy intake (kcal/d) of male grizzly bears (*Ursus arctos*) housed at San Diego Zoo in 2016.

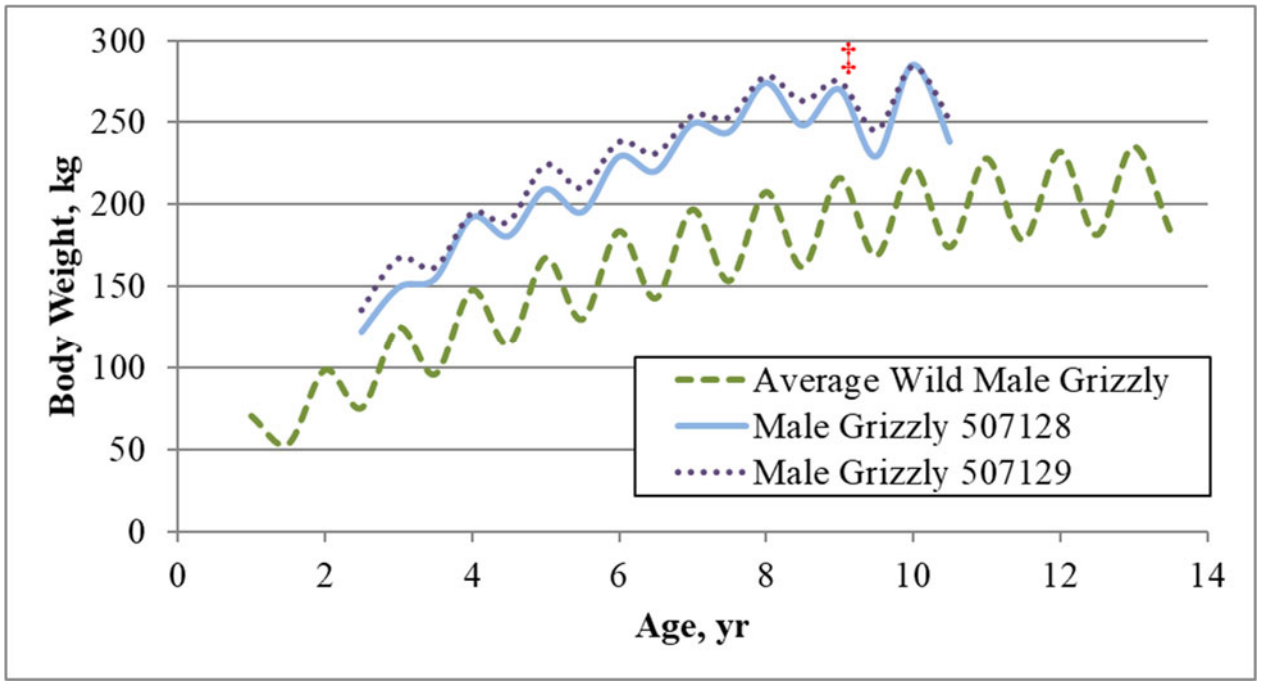

Figure 3. Growth of male grizzly bears (*Ursus arctos*) housed at San Diego Zoo compared to values adapted from Kingsley *et al*. (1983) for *in situ* male grizzly bears from northern Canada. Initiation of diet management change denoted by: ‡.

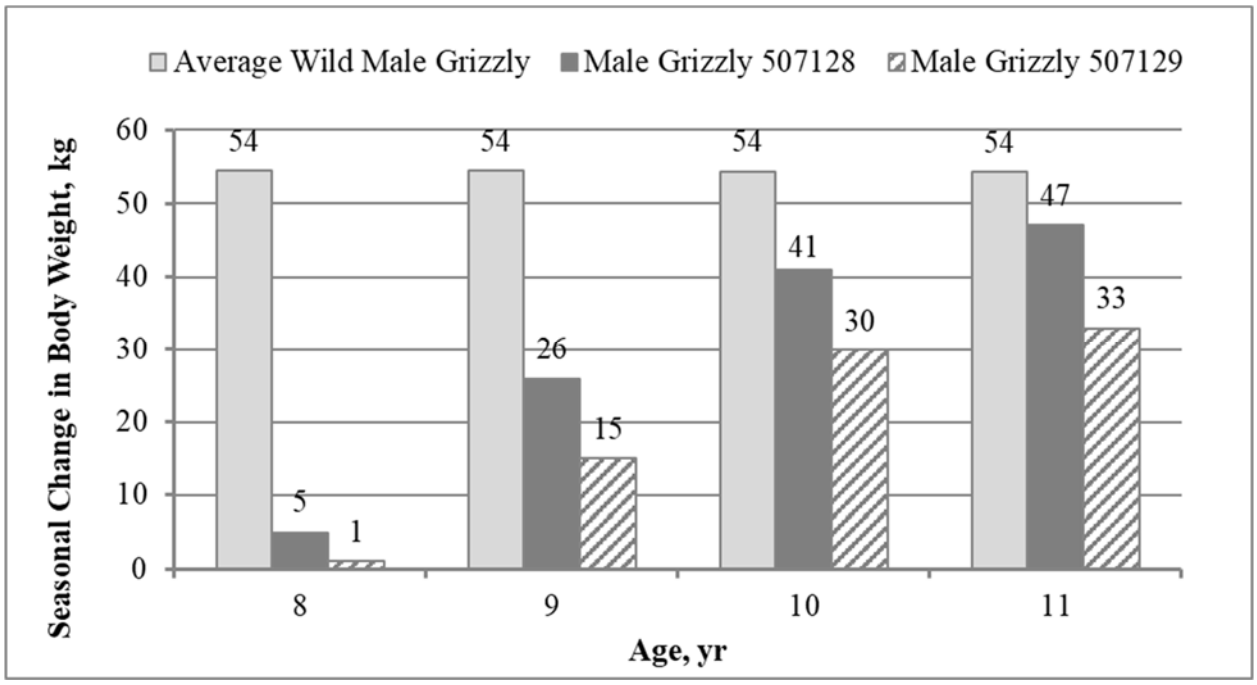

Figure 4. Seasonal change in body weight (kg, fall to spring) of male grizzly bears (*Ursus arctos*) housed at San Diego Zoo compared to values adapted from Kingsley *et al*. (1983) for *in situ* male grizzly bears from northern Canada. Dietary management change implemented from 10 to 11 y-old.

FROM FARM TO FANG: SOURCING CARCASSES FOR CARNIVORES

Katherine Kerr, PhD[1]*, Jessica Sheftel, BS[1], Meg Sutherland-Smith, DVM[2], Meredith Clancy, DVM[3], Lauren Howard, DVM[3], and Andrea Fidgett, PhD[1]

[1]San Diego Zoo Global, San Diego, CA 92112, USA
[2]San Diego Zoo, San Diego, CA 92112, USA
[3]San Diego Zoo Safari Park, Escondido, CA 92027, USA

Abstract
Incorporating large whole prey items into the diet of carnivores (big cats, condors, and more) can provide complete nutrition – from the meat, bones, organs, and even fur, as a source of fiber – with the beneficial effects of stimulating activity and promoting species-specific, naturalistic feeding behaviors. San Diego Zoo Global is evaluating potential addition of large whole prey items into the dietary management and welfare plans of carnivores at our institution. The process from planning to initiation to evaluation will be discussed, including key topics such as supplier selection, logistics, and discussions regarding potential risks.

BLOOD FATTY ACID PROFILES OF JUVENILE WILD GREEN TURTLES (*CHELONIA MYDAS*) AND KEMP'S RIDLEY TURTLES (*LEPIDOCHELYS KEMPII*)

Elizabeth A. Koutsos, PhD[1]*, Jb Minter, MS, DVM[2], Kimberly Ange-van Heugten, PhD[3], Johanna Mejia-Fava, PhD, DVM[4], Craig A. Harms, PhD, DVM[5]

[1]Koutsos Consulting LLC, 2084 Toad Hollow Trail, Apex, NC 27502, USA
[2]North Carolina Zoo, 4401 Zoo Pkwy, Asheboro, NC 27205, USA
[3]Department of Animal Science, North Carolina State University, Raleigh, NC 27695-7621, USA
[4]Animal Necessity LLC., New York, NY 10018, USA
[5]College of Veterinary Medicine and Center for Marine Sciences and Technology, North Carolina State University, Morehead City, NC 28557, USA

Abstract

Blood fatty acid profiles of wild and captively managed animals are of interest because fatty acids directly impact numerous physiological functions including immune function and reproductive success (Holman, 1971, Fritsche, 2006) and can be modified by diet (Field and Clandinin, 1984; Suedmeyer and Dierenfeld, 1998). Newer methods for fatty acid analysis can provide information about long term fatty acid status of an animal in one drop of whole blood (Armstrong *et al*. 2008; Bailey-Hall *et al*. 2008). This technology was applied to understand the fatty acid nutrition of wild caught juvenile green turtles (*Chelonia mydas*; $n = 9$, 6 females, 3 males) and Kemp's ridley turtles (*Lepidochelys kempii*; $n = 8$, 6 females, 2 males). Juvenile green turtles are carnivorous, feeding on crustaceans, jellyfish, and ctenophores (Bolten, 2003). Then they shift to a herbivorous diet, focusing on seagrasses and algae (Cardona *et al*. 2010; Jones and Seminoff, 2013). Kemp's ridley turtles, in contrast, are much more carnivorous throughout their life, feeding on crabs and other benthic invertebrates (Shaver, 1991). The predominant fatty acids in the blood of both turtle species were 18:1ω9 oleic acid, 16:0 palmitic acid, 20:4ω6 arachidonic acid, 18:0 stearic acid and 16:1ω7 palmitoleic acid. Green turtles had lower levels of ω7 fatty acids and 20:5ω3 eicosapentaenoic acid and higher levels of 18:2ω6 linoleic acid, 18:3w3 linolenic acid, and 22:5ω3 docosapentaenoic acid compared to Kemp's ridley turtles, ($P < 0.05$ for each). Despite predicted low dietary 20:4ω6 arachidonic acid levels, blood levels were readily detected, suggesting that both species can convert linoleic acid to arachidonic acid. These data can be used to assess and guide nutrition programs for sea turtles under human care.

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BRINGING A NEW LEVEL OF GREEN TO THE NUTRITION CENTER – THE ROAD TO ZERO WASTE CERTIFICATION AT DISNEY'S ANIMAL KINGDOM

Shannon E. Livingston, MSc[1]*, Debbie Weber, BS[1], Elisabeth Brunk[1], and Eduardo V. Valdes PhD[1]

[1]Department of Animal Health, Disney's Animals, Science, and Environment. 1180 N. Savannah Circle, Bay Lake FL 32830, USA

Abstract

The Animal Nutrition Center at Disney's Animal Kingdom received Zero Waste Certification from the US Zero Waste Business Council at Platinum Level in 2016. Inspired by the Walt Disney Company's environmental stewardship goal to divert 60% of waste from landfills and incineration by 2020 as well as the Zero Waste Certification of the Land pavilion at Epcot, the Animal Nutrition Center started the journey to Zero Waste in 2015. After an initial measurement of 95% landfill diversion due to long-standing internal practices, the area began quarterly measurements to determine the baseline value for one year. Each quarter, measurements of total trash generated and amount of trash diverted from landfill were reported for seven consecutive days. The average percentage of trash diverted from landfill recorded for each quarter was over 95%. These values, as well as documented efforts to reduce, reuse, and recycle, helped to qualify the Animal Nutrition Center for Platinum level Certification, which was awarded in September of 2016.

Introduction

The Animal Nutrition Center at Disney's Animal Kingdom has always aimed to limit the amount of waste going to landfill. Diets are prepared in reusable containers, washable hand towels are used in place of disposable paper towels, wooden pallets and cardboard boxes are reused in addition to recycling and other programs designed to divert items from landfill. Zero Waste Certification by partners at the Land (at Epcot) inspired the nutrition team to pursue their own certification with the US Zero Waste Business Council. Zero waste certification with the US Zero Waste Business Council is based on a multitude of criteria. In order to begin the certification process, an operation must record trash diversion from landfill and incineration over a 7 day period and average over 90% diversion rate. Once the 90% diversion rate is reached, measurements must be conducted quarterly for a one year period with diversion rates maintained over the 90% threshold.

Methods

The Animal Nutrition Center has employed strategies to reduce their environmental impact for many years. Diets are prepared in reusable containers to limit the use of plastic bags. Food waste is picked up daily and composted producing energy and fertilizer. The nutrition center lab reuses shipping boxes. Many items are washed and donated to groups like A Gift for Teaching who reuse them for school projects. Bulk towels are used to reduce the need for paper towels. Wooden shipping pallets are stored and returned to vendors for reuse in future shipments. Existing recycling programs were utilized. A seven day measurement of trash and landfill/incineration diversion was conducted. All waste was weighed and the amount diverted from landfill or incineration was calculated to be greater than 90% over the seven day period.

In order to move towards certification, the Animal Nutrition Center worked closely with the Disney Environmental Integration Team and The Land pavilion at Epcot, who had just received Platinum level Zero Waste Certification, to learn best practices. All garbage and recycling containers from throughout the Animal Nutrition Center were removed and replaced with a centrally located recycling station. The Animal Nutrition Center team was trained to sort waste according to its waste stream. Items were sorted to be reused, recycled, or incinerated. An outside company was employed to find non-landfill and non-incineration options for some of the waste products. The Animal Nutrition Center worked with suppliers and vendors to find packaging options that resulted in fewer items going to landfill/incineration. Quarterly waste measurement continued with the waste diversion rate recorded at 95%, 98%, 95%, and 97%, after which time the application process for Zero Waste certification was started. The application consists of 15 categories with a possible 81 points. The 15 categories include redesign, reduce, reuse, compost, close the loop, recycle, reporting, diversion from landfill, zero waste purchasing, leadership, training, zero waste analysis, upstream management, hazardous waste prevention, and innovation. After submittal of the Animal Nutrition Center application and supporting documentation, the US Zero Waste Council conducted an on-site inspection. The level of Zero Waste certification is based on the score resulting from the application; bronze is 31-37 points, silver is from 38-45 points, gold from 46 to 63 points, and platinum from 64 to 81 points.

Results

The Animal Nutrition Center scored 72 points on the application and was awarded Platinum level Zero Waste Certification.

Discussion

The Animal Nutrition Center continues to work to limit the amount of waste going to landfill/incineration. A number of other teams within the Disney Animals, Science and Environment line of business have since been inspired to start their own Zero Waste programs, further helping to reduce the overall amount of items going to landfill/incineration from Disney's Animal Kingdom.

The Zero Waste Certification program may not be the best option for everyone as there are costs involved. Regardless, there are benefits to reducing the amount of waste going to landfill. Overall operation costs can be reduced and efficiencies found. Alternative disposal streams can be discovered which are beneficial to multiple parties. Some items can be collected and donated to organizations that use them for charitable benefit like aluminum can tabs for Ronald MacDonald House or hard plastic caps for Caps of Love which are sold and the money used to buy wheelchairs for children. Some areas recycle rubber-soled shoes which are recycled into soft surfaces for play grounds. The Animal Nutrition Center saves empty plastic strawberry and blueberry containers which are used for art projects by A Gift for Teaching. The Animal Nutrition Center worked with vendors to reduce disposable packaging and utilize reusable containers such as large plastic bins for rodent delivery or small cambros for mealworm delivery instead of disposable cardboard boxes. In addition to decreased packaging for disposal, time efficiencies were created as the mealworms arrive prepackaged in the desired amounts and rodents no longer need to be sorted into bins. Other vendors have changed packaging from black plastic wrap which is non-recyclable to clear plastic wrap that can be recycled. A feed manufacturer has not only started a pilot program to recycle poly woven pellet bags, they now have a sustainability department as well.

Conclusion

The response from nutrition center cast, other Animal Kingdom teams, other partners, and vendors has been positive. Once people understood the reason for sorting waste and how to do it, compliance has been strong, resulting in a contamination rate of less than 0.03%. The interest and motivation has spread throughout the Animals, Science, and Environment line of business to other partners within Walt Disney World as well as to vendors that supply the Animal Nutrition Center helping to further reduce the environmental impact on our planet.

Acknowledgements

The Platinum Level Zero Waste certification for the Animal Nutrition Center at Disney's Animal Kingdom could not have been accomplished without the hard work and support of many people. Debbie Weber and Liz Brunk are the ANC champions for Zero Waste - their dedication and efforts have inspired the rest of the nutrition team as well as many other animal care teams to decrease the amount of waste going to landfill. The Animal Kingdom leadership team has been a strong supporter of the Zero Waste efforts. The authors also recognize all the vendors that have made changes to their operations in order to help our efforts for reducing waste and environmental impact. Thank you to Katie Sullivan and Scott Williams for aid in edits.

DOING MORE WITH LESS AT BUSCH GARDENS TAMPA BAY: IMPROVING COMMISSARY EFFICIENCY

Clifton Martel, MBA and Heidi Bissell, PhD*

SeaWorld Parks & Entertainment, Dept. of Zoological Operations, 3605 E. Bougainvillea Ave, Tampa, FL 33612, USA

Abstract

Maximizing the efficiency of food preparation is the goal of every commissary operation. At Busch Gardens Tampa Bay, a number of process improvements have allowed us to take on additional projects and reduce our labor costs. These include: a thorough re-design of our diet cards to minimize eye movement, maximize clarity, and reduce mistakes; pre-bagging dry goods to minimize traffic in the kitchen; a computerized system that prints specialized stickers that increase food safety and preparation accuracy, specialized reports that allow us to prepare and pull exactly what is needed the next day without wasting food, and a new touchscreen system that we hope will continue the process of error-reduction and speed-enhancement. Underlying all of this has been a philosophy of "let's just try it" that has given us the flexibility to test out changes, discard ones that didn't work, and adopt the ones that do.

EFFECT OF DIFFERENT PROTEIN LEVELS ON THE PERFORMANCE AND APPARENT PROTEIN DIGESTIBILITY OF ORPHAN CALVES OF AMAZONIAN MANATEES (*TRICHECHUS INUNGUIS*)

Pierina Mendoza, BSc[1]*, Rony Riveros, BSc[1], and Carlos Vilchez, PhD[1]

[1]Department of Nutrition, Universidad Nacional Agraria la Molina, La Molina Av. s.n., Lima, Peru

Abstract

The objective of this study was to evaluate the effect of four dietary crude protein (CP) levels, by mixing two commercial milk replacers, on the performance and apparent protein digestibility in captive orphan calves of Amazonian manatees (*Trichechus inunguis*). Four individuals (two males and two females), with an average weight of 28.95 kg and an average age of 8.75 months, were used in the study. The dietary treatments were: Diet 1 (7.22 g CP), Diet 2 (9.77 g CP), Diet 3 (12.25 g CP) and Diet 4 (14.88 g CP). Each animal received one of each diet during 14-day period with a 7-day adaptation period between diets. Feed intake, weight gain and feed conversion were recorded. Chromium oxide was used to determine apparent protein digestibility; additionally, the protein content of manatee's milk was determined. Registered data was analyzed using a 4x4 Latin Square Design and mean comparisons were performed using the statistical software IBM SPSS 24.0. The results showed that feed intake and weight gain were not influenced ($P > 0.05$) by the dietary treatments. However, the lowest feed conversion corresponded to Diet 3, which was different ($P < 0.05$) from Diet 1, but it was not ($P > 0.05$) from Diet 2 or Diet 4. The highest apparent protein digestibility corresponded to Diet 4. The protein content of manatee's milk was 9.70%. In conclusion, orphan calves of manatees can be fed with different proportions of commercial milk replacers without affecting their performance.

Introduction

The Amazonian manatee (*Trichechus inunguis)* is an aquatic herbivorous mammal belonging to the Sirenia Order, of high ecological value as it contributes to the fertilization of water to produce plankton. Endemic species of the Amazon watershed (Brazil, Colombia, Ecuador and Peru; Best, 1984; Rosas, 1994; Domming, 1981; Timm *et al*., 1986; Rosas, 1991) utilize sweet water as exclusive habitat (Best, 1984; Husar, 1977; Rosas, 1994).

Several risk factors threaten the current population; therefore, the manatee is cited in Appendix I of CITES (The Convention on International Trade in Endangered Species of Wild Fauna and Flora; Soini *et al*., 1996; Silva and Montes, 2014; MINAM, 2014) and as vulnerable by IUCN (International Union for Conservation of Nature; IUCN, 2016). Currently, in Peru, it is considered a "protected Amazonian species" under the Peruvian Amazonian Regulation of Fishing Regulations (Ministerial Resolution Nº 147-2001-PE, 2001) and cataloged "in danger of extinction at national level" (Supreme Decret Nº 034-2004-AG, 2007).

Hunting has led to an increase in the number of orphan infant offspring; therefore, rescue and conservation strategies have been established (Soto, 2007). It is necessary to investigate and establish nutritional requirements for appropriate alimentary management of animals in captivity at rescue centers. Reports of hand-rearing manatees are few and consist of variations of breastmilk

(Best *et al*., 1982; Rodriguez Chacon *et al*., 1999; Barbosa, 2011, Maduro, 2014) with the aim of approaching nutritional requirements of the species, which are still unknown.

Among the different nutrients, protein is a macronutrient that stands out for its great importance in tissue regeneration, muscle mass development, enzyme production, and hormones. Determination of the protein requirement of a species would not only allow adequate development of the animal but also allow confirmation of an adequate contribution of this nutrient in the diet. This would thereby avoid nutritional deficiencies, anemia, and excesses which would increase nitrogen removed in urine and feces increasing eutrophication of the rivers where the animal lives.

The objective of the present study was to evaluate the nutritional profile of the Amazonian manatee (*Trichechus inunguis*) lactating in captivity, fed with four different levels of protein, considering the productive performance, digestibility, and nutritional composition of milk as a reference of the protein requirement of the species.

Materials & Methods

Experimental animals and location

A total of 4 previously identified lactating manatees (2 males and 2 females), with an average weight of 28.95 ± 1.36 kg and an age of about 8.75 months, were randomly distributed in individual pools provided with shade and a water filtration system. The trial was carried out at the facilities of the Amazon Rescue Center (CREA) in the city of Iquitos-Peru (Figures 1 and 2).

The daily management consisted of monitoring the ambient temperature and water, maintained at a temperature of no less than 23°C.

Treatments

Four diets were evaluated (different proportions of two lactose-free dairy substitutes) to obtain the proposed nutritional contribution. The milk replacers used were a specialized formula (ZMM) and another commercial formula (NLF). The proportions of the milk replacer and the calculated crude protein content for each diet are presented in the Table 1.

The study was separated into 4 experimental periods (14 days each), with intervals of 7 days of adaptation between each experimental period. During each experimental period, the four diets were evaluated in a different animal (Figure 3). Feeding was performed daily using the following feed protocol:

- During evaluation periods 1 and 2, 1 liter per animal (4 rations of 250 ml/ration/day).
- During periods 3 and 4 supply was increased to 1.5 liters per animal (6 rations of 250 ml/ration/day) in response to the increased requirement of consumption for the growth of animals. The amount of feed offered and residual was measured with a graduated cylinder ± 1 ml and of 250 and 50 ml of capacity (Figure 4).

Response variables

Zootechnical performance

At the beginning of the experiment, individual weights of the four infants were recorded before consumption of the diet was evaluated. Throughout the experiment the weight at the beginning and end of each experimental period was recorded with an electronic balance of 200 kg of capacity

and ±1 g of sensitivity. The variables obtained from each diet per period (14 days) were: body weight gain (BWG), total feed intake, and feed conversion rate (FC; Figure 5).

Apparent protein digestibility

Digestibility observational evaluation was performed using an inert marker of chromium oxide (Cr_2O_3) added in each diet. Between the fourth and fifth evaluation days of the first and third periods, 1.19% of chromic oxide (0.5 g) DM, was added for one day in each nipple (Figures 6-8).

For a significant sample collection, animals were continuously monitored to avoid loss of fecal samples through disintegration in the water and/or coprophagy. The difference between consumed and excreted determined the coefficient of apparent digestibility.

Dry matter digestibility was calculated through the following equations (Harshaw, 2012):

$$\text{DMD diets} = 100 - (100 * \frac{\%\ Cr2O3\ \text{in feed}}{\%\ Cr2\ O3\ \text{in feces}})$$

$$\text{DMD nutrients} = 100 - (100 * \frac{\%\text{nutrient in feces x }\%\ Cr2O3\ \text{in feed}}{\%\text{nutrient in feed x }\%\ Cr2\ O3\ \text{in feces}})$$

Representative samples of the diets offered were collected at the end of the experiment, taking total samples of 100 g for each diet evaluated. Samples from the diets and feces collected from each diet were stored in airtight bags and transported for determination of moisture content, dry matter and protein (Kjeldhal, 1883). In addition, the content of chromium in feces was determined (Figure 9).

Milk sample collection

In the semi-upland area of the CREA, fishing net was used to carry out physical containment of the lactating mother (Figures 10-14). Once near the shore, the animal was placed on a care stretcher. The animal under observation was a first-time mother, found in its second semester of lactation. After disinfection of the mammary gland, the animal was milked manually, and the sample placed in a recently sterilized autoclavable 100 ml capacity vessel (Figure 15-16). After milk collection, the sample vessel was placed in a cooler to be transported and stored in refrigeration at 4°C until analysis of protein content.

Statistical analysis

A Latin Square Design (LSD) was used with four treatments and four replicates per treatment (4x4). Data were subjected to a normality analysis and subsequently to a one-way ANOVA by the general linear model (GLM) procedure and comparison of means of the diets for the final weight, weight gain, feed intake and feed conversion rate by the Duncan test. A *P*-value less than 0.05 were considered the level of significance. The statistical software IBM SPSS 24.0 (IMB Corporation. Armonk, NY) was used.

Results

Zootechnical performance

Table 2 describes results obtained by the Duncan test. No significant differences were observed between the four diets for final weight, weight gain, or food consumption ($P > 0.05$). In contrast, a significant difference was found in cumulative feed conversion ($P < 0.05$) with diet 3 of minor feed conversion (6.22) and diet 1 the highest feed conversion (9.16). Diet 3 presented higher food efficiency compared to others, consumption was reduced and animal weight increased.

Apparent protein digestibility
Observational results of total protein and dry matter digestibility of the four diets evaluated are reported in Table 3. High digestibility of dry matter (63.64-84.27%) and total protein (77.08-94.62%), nutrient of greater utilization of the lactating animal, were obtained. Observational comparison of the different diets suggests that Diet 4 presented the highest digestibility of dry matter and crude protein.

Analysis of protein content of milk.
Amazonian manatee milk has a liquid appearance, slightly creamy, homogeneous, and quite viscous with an opaque white coloration. Collected milk samples left standing formed a layer of fat on the surface. An average of 9.70% protein content of the Amazonian manatee milk sample was obtained in the second semester of lactation.

Discussion
Zootechnical Performance
A range of 0.9-1.21 kg/wk weight gain was recorded, in agreement with average weekly weight gains reported in previous studies performed in similar conditions developed by Best *et al*. (1982), Rodriguez Chacon *et al*. (1999), and Maduro (2014). Although there was no significant statistical difference in body weight gain between the diets, it was observed that diet 3 (2.42 kg per 14 days) showed some improvement in body weight gain, more than half a kilo higher than the weight gain obtained by diet 1 (1.80 kg/period), the diet that presented the lowest weight gain.

The resulting daily feed intake was 1.05-1.11 kg of milk per animal, which was much lower than reported by Best *et al*. (1982). However, authors such as Rodriguez Chacon *et al*. (1999a) and Maduro (2014), also obtained daily food consumption lower than that reported by Best et al. (1982), similar to those presented in the present study. Although average food consumption was lower than previously reported, weight gains were similar to studies mentioned above. It is suggested this is because inputs from the diets supplied were possibly more usable than inputs used in dairy formulas of previous studies.

Water temperature is an important factor that may affect food consumption. Temperatures lower than 20°C and higher than 31°C may result in depression and/or loss of appetite in infants, as they are more susceptible that adults (Vanoye, 2002). In the present study, water temperature and pH were recorded and no variations were observed that would affect the test. Average ambient temperature was 28.8°C, and average water temperature was 27.4±0.5°C, which is within the temperature range of comfort for the young.

The feed conversion obtained in the present work from 6.22 to 9.16 was lower than that reported in previous studies by Rodriguez Chacon et al. (1999a) and Maduro (2014). In this aspect, significant statistical differences were found, Diet 3 resulted in the better feed conversion

($p<0.05$) suggesting greater dietary efficiency of the diet. With the provision of a more nutritious food, frequency of feeding during the day can be reduced, reducing human contact with the animals. This may have a positive influence on the reintroduction programs of these animals to the wild.

Apparent protein digestibility

Manatees appear to be more efficient than other wild mammals in their ability to digest protein, with the cecum and colon being the primary sites where manatees digest the protein and lipid components of the feed (Burn, 1986). This was evidenced by the fact that the average protein content in feces (13.74%) was lower by approximately 47.68% with respect to the average crude protein content of the diet (26.26%). Crude protein content in the feces of the four diets evaluated presented independent variations of the crude protein content of the diet. Crude protein content in feces was expected to increase as crude protein intake from diets increased; however, diets 2 and 4 had lower crude protein values in feces (10.57% and 12.11%, respectively) compared with diet 3, which showed a higher value (17.06%) despite having a lower contribution than diet 4. This could be due to other factors such as digestibility and amino acid profile of different diets but not the amount of crude protein, which was evidenced when determining the protein digestibility coefficient of each of the diets, diets 2 and 4 the highest coefficients of protein digestibility (85.59% and 94.62%), respectively.

In direct comparison, diet 4 showed the highest protein digestibility coefficient (94.62%); however, it did not present the best productive performance which suggests that it has highly digestible protein sources but does not take into account the bioavailability of the amino acid profile of diets.

Among the four diets evaluated, diet 4 had the highest dry matter digestibility coefficient (84.27%), which is similar to the range (68-82%) reported by Harshaw (2012) in adult *T. manatus latirostris*. However, a high digestibility is not necessarily an indicator of a suitable diet for the species, since its nutritional content could be highly fermentative causing changes in the gastro-fecal pH, alteration of the intestinal biota, and accelerated growth rhythm with alterations such as obesity without frequent monitoring (Harshaw, 2012).

In another context, the present preliminary digestibility assessment remains a matter of study, in response to the scarce bibliographic information available. The high values of apparent digestibility reported by Harshaw (2012) and the present study are not conclusive evidence of the physiological condition of the animal or of the feed offered. This species practices the coprophagy habit as a strategy to maximize nutrient utilization (Rodriguez Chacon *et al*., 1999b), a factor that affects digestibility, therefore it is suggested to make an adjustment in the method of evaluation of digestibility.

Analysis of protein content of milk

The difficulty of obtaining sufficient milk volume for analysis occurred in previous studies performed with *T. manatus* by Bachman and Irvine (1979) and Vergara et al. (2000). Likewise, the observed physical characteristics are in agreement with previous studies carried out with manatee milk by Barbosa (2011), for *T. inunguis*; and Pervaiz and Brew, (1986), for *T. manatus latirostris*.

The protein content of milk obtained in the present study (9.7%) was found within the range of 4.24-10.47% reported by the only previous report in this species (Barbosa, 2011). When compared to similar studies performed on two subspecies of the marine manatee, one can observe slight superiority to the protein content of Florida manatee milk (*Trichechus manatus latrirostris*; 6.9-9.0%; 9.65% y 9.7%) reported by Bachman and Irvine (1978), Pervaiz and Brew (1986), and Worthy (1990), respectively. In addition, the milk protein content is greater than that reported by Verara *et al*., (2000) for the manatee of the Antilles (*Trichechus manatus manatus*), 5.25%. The difference in protein content, in addition to being influenced by the species, may also be related to factors such as: type of food offered to the animal in captivity, condition of the animal from which the sample was extracted (live or dead animal), condition in which the animal lives (captivity or free life), and stage of lactation, since previous studies have shown decreasing levels of total lipids and proteins with the increase of the lactation phase (Pervaiz and Brew, 1986; Eichelberger *et al*., 1940).

Previous studies have reported the following milk protein concentrations of aquatic mammals: baleen whale (*Suborder Mysticeti*): 9-15% and toothed whale (*Suborder Odontoceti*): 8-11% (Oftedal, 1997); pink river-dolphin (*Inia geoffrensis*): 9.6% (Rosas and Lehti, 1996), bottlenose dolphins (*Tursiops truncatus*): 12.2% (Pervaiz and Brew, 1986), spotted dolphin (*Prodelphinus plagiodon*): 9.4% (Eichelberger *et al*., 1940), humpback dolphin (*Sousa plumbea*): 11.30% and common dolphin (*Delphinus delphis*): 10.30% (Peddemors *et al*., 1989); respectively. The range milk protein in other cetaceans varies from 7.1 to 12.8%. Compared to the milk protein content of the Amazonian manatee (*Trichechus inunguis*), a high similarity is observed with the aquatic mammals mentioned above, evidencing one of the most common characteristics of the milk of this group of animals: the high protein content in milk (Jenness and Sloan, 1970).

The transfer of protein for breeding is much greater than given in other terrestrial herbivorous mammals such as the Brazilian tapir (*Tapirus terrestris*) with a content of 4.4% (Jensen, 1995), mountain zebra (*Equus zebra*) with 1.6% protein (Jensen, 1995), and domestic animals with 3.5% and 3.3% protein in cattle (FAO, 2017) and domestic goats (Boza and Sanz, 1997), respectively. Barbosa (2011) explains that higher concentration of protein in aquatic mammals may be due to the increased need for oxygen retention for diving in addition to the fact that proteins are essential nutrients for the formation of structures and blood cells responsible for the transport of oxygen.
Comparison of the four diets, relative to protein content, productive performance, and digestibility, indicated that diet 3 (12.25 g CP) presented the best feed consumption and feed conversion due to slightly higher protein intake. It was also observed that diet 4 (14.88 g CP), despite presenting a high digestibility of the protein, was not accompanied by the better productive performance. This may be due to the profile of amino acids and to an excess of protein when compared to breast milk.

Conclusions

1. With the exception of feed conversion, zootechnical performance of the experimental animals was not significantly influenced by the dietary treatments.

2. The apparent protein digestibility varied from 77.08 to 94.62 %, and the highest value corresponded to diet 4 (14.88 g CP).

3. The crude protein content of Amazonian manatee´s milk was 9.70%.

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Table 1. Composition and crude protein content of experimental diets fed to Amazonian manatees (*Trichechus inunguis*).

		Diet			
Item	**Unit**	**1**	**2**	**3**	**4**
ZMM	%	25	50	75	100
NLF	%	75	50	25	0
Mix of ZMM + NLF fed/animal/d	g	42	42	42	42
Crude protein of ZMM + NLF Mix	%	17.19	23.25	29.16	35.43
Amount of crude protein fed/animal/d	g	7.22	9.77	12.25	14.88

Table 2. Mean initial body weight, final weight, body weight gain, feed intake, and cumulative feed conversion rate of Amazonian manatees (*Trichechus inunguis*) fed four experimental diets for 14 days of evaluation.

		Diet[2]			
Measurements[1]	**Unit**	**1**	**2**	**3**	**4**
Final body weight, kg	kg	34.17[a]	34.16[a]	34.54[a]	34.68[a]
Body weight gain 14 d-period, kg	kg	1.80[a]	2.28[a]	2.42[a]	2.16[a]
Total feed intake in 14 d-period, kg	kg	15.34[a]	15.57[a]	14.73[a]	14.69[a]
Feed conversion ratio	kg:kg	9.16[a]	6.95[ab]	6.22[b]	7.38[ab]
Final body weight, kg	kg	34.17[a]	34.16[a]	34.54[a]	34.68[a]

[ab]Different letters in the same row are statistically different values ($P < 0.05$)
[1]Values for each diet represent the average of the 4 replicates
[2]Diets: D1 (7.22 g CP), D2 (9.77 g CP), D3 (12.25 g CP), and D4 (14.88 g CP)

Table 3. Dry matter and crude protein digestibility of four diets fed to Amazonian manatees (*Trichechus inunguis*).

	Digestibility (%)	
Diet[1]	**Dry matter**	**Crude Protein**
1	74.13	77.08
2	63.90	85.59
3	63.64	78.73
4	84.27	94.62

[1]Diet: D1 (7.22 g CP), D2 (9.77 g CP), D3 (12.25 g CP), and D4 (14.88 g CP)

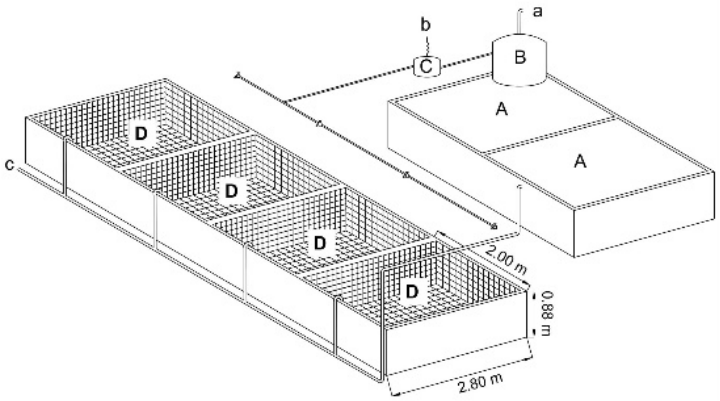

Figure 1: Facilities of the Quarantine Zone of the Amazon Rescue Center (4 pools of 2.79 m long x 2.01 m wide x 0.88 m deep), and schema of distribution in the area (A: water reserve pools; B: treated tank for the water filtration system; C: water thermoregulation thermos; D: individual pools; a: water supply; b: electrical supply: c: lead of water.

Figure 2. System for filtering and renewing water from a treated tank (B).

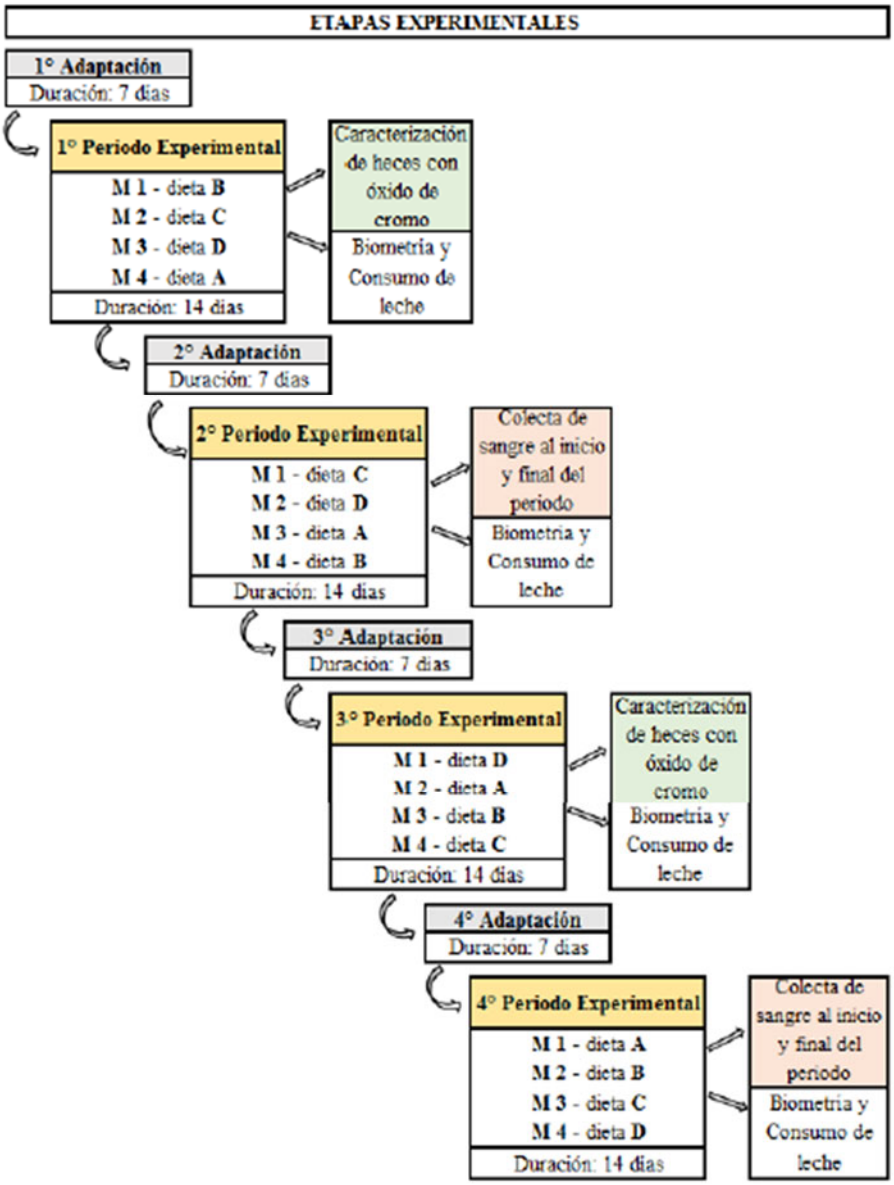

Figure 3. Scheme of the stages of the experiment, duration of the periods and activities.

Figure 4. Nipples and feeding bottles used for the daily feeding of Amazonian manatees (*Trichechus inunguis*).

Figure 5. Periodical and individual weighing of Amazonian manatees (*Trichechus inunguis*) by physical condition.

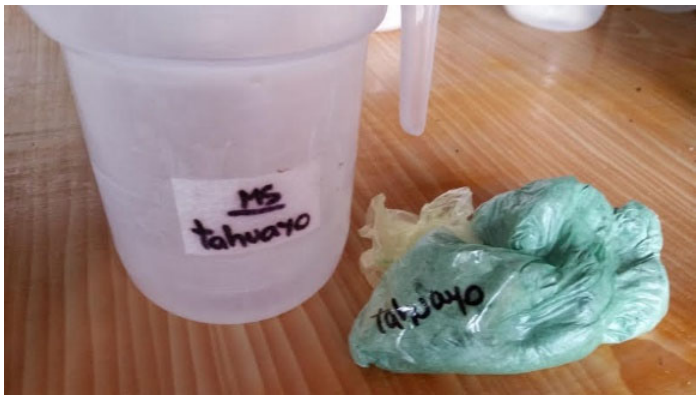

Figure 6. Use of chromic oxide as an external marker and mixed "in dry" with dietary treatments fed to Amazonian manatees (*Trichechus inunguis*).

Figure 7. Chromic oxide mixed and diluted in the respective nipples prior to feeding Amazonian manatees (*Trichechus inunguis*).

Figure 8. Milk replacer with the external marker offered to Amazonian manatees (*Trichechus inunguis*).

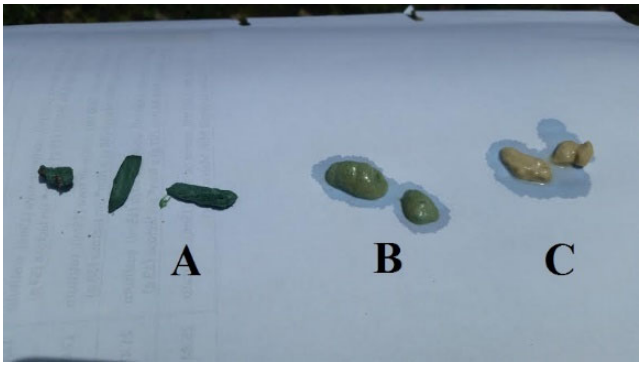

Figure 9. Difference in coloration of marked (A), partially marked (B), and unmarked feces (C) of Amazonian manatees (*Trichechus inunguis*) with the external marker.

Figure 10. Artificial body water in the Zone of Semi-captive.

Figure 11. Displacement of the Amazonian manatee (*Trichechus inunguis)* mother towards the riverside for its subjection.

Figure 12. Cave feeding of Amazonian manatee (*Trichechus inunguis*) with breast milk in semi-captivity.

Figure 13. Physical containment of the lactating Amazonian manatee (*Trichechus inunguis*) mother.

Figure 14. Manual milking of the Amazonian manatee (*Trichechus inunguis*) under evaluation on the care stretcher.

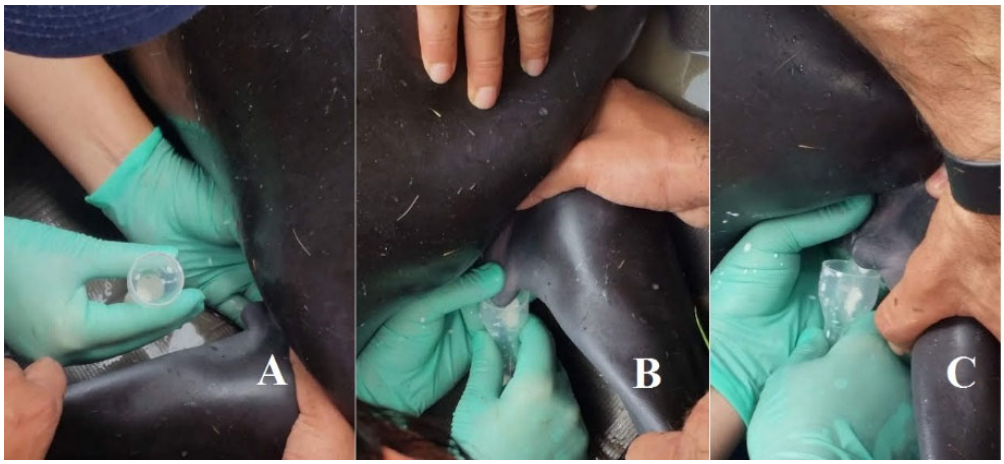

Figure 15. Stimulation of the Amazonian manatee (*Trichechus inunguis*) mammary gland through massages (A), manual milked (B), and prior to milking (C).

Figure 16. Storage of the collected Amazonian manatee (*Trichechus inunguis*) milk sample in a sterile container.

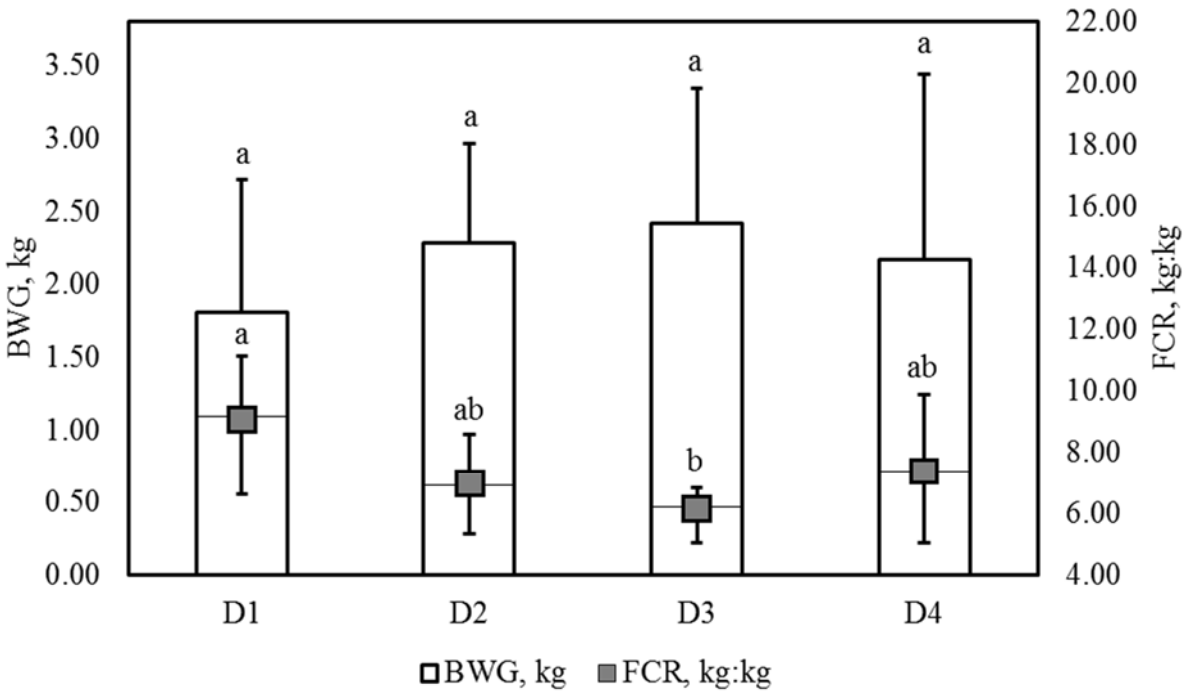

Figure 17: Comparative diagram of the body weight gain (BWG) and feed conversion ratio (FCR) for the dietary treatments fed to Amazonian manatees (*Trichechus inunguis*).

CASE STUDY: IRON IN BLACK RHINOCEROS (*DICEROS BICORNIS*) DIETS: THE IMPACT OF PASTURE

Jasmyn Mimiko, MS[1]*, Elizabeth Stringer, DVM, DACZM[2], and Jennifer Parsons, PhD[1]

[1]Nutrition Department, Denver Zoological Foundation, 2300 Steele St., Denver, CO 80205-4899, USA
[2]Veterinary Medicine Department, Denver Zoological Foundation, 2300 Steele St., Denver, CO 80205-4899, USA

Abstract
Over the course of a year, serum ferritin and iron in a black rhinoceros (*Diceros bicornis*) increased over time, despite a prescribed diet low in iron. To identify additional sources of iron intake, vegetation in the enclosure was analyzed for iron content. Iron concentrations in enclosure samples averaged 736 ppm, well in excess of maximum recommended total-diet concentrations of 300 ppm. The rhinoceros consumes pasture grass in the enclosure, which may be leading to high iron intake and subsequent internal accumulation. We recommend that institutions holding iron-sensitive rhinoceros' species test any vegetation available to the animals and not only prescribed feeds.

MACRONUTRIENT COMPOSITION OF MILK FROM AN ASIAN RHINOCEROS (*RHINOCEROS UNICORNIS*) ACROSS LACTATION

Katie L. Murtough, MS, MPP[1,2]*, Michael L. Power, PhD[1], Ann Ward, MS[3]

[1]Nutrition Laboratory and Conservation Ecology Center, Smithsonian Conservation Biology Institute, National Zoological Park, 3001 Connecticut Ave NW, Washington DC 20008 USA
[2]University of Maryland College Park, College Park MD, 20742, USA
[3]Nutritional Services, Fort Worth Zoo, 1989 Colonial Parkway, Fort Worth TX, 76110, USA

Introduction

Milk composition is a critical aspect of all female mammalian reproductive strategies. The first and usually sole food over an extended time period for mammalian neonates is mother's milk. The macronutrient composition of milks from different species can vary widely (Oftedal and Iverson, 1995; Skibiel *et al*., 2013). In this study, we present data on the macronutrient composition of milk from an Asian rhinoceros (*Rhinoceros unicornis*) cow collected between calf ages 4 and 9 months and compare to the composition of milks of other large terrestrial herbivores.

Materials & Methods

Milk samples were collected by manual expression from a single Asian rhinoceros cow at the Fort Worth Zoo from day 123 through day 284 postpartum (n = 14). Samples were assayed for dry matter (DM), fat, sugar, crude protein (CP), and ash using standard methods that have been validated at the Nutrition Laboratory of the Smithsonian National Zoological Park and performed on milk samples from about 200 species of mammals (Hood *et al*., 2009). Briefly, for DM, milk samples were aliquoted, weighed, and dried in a forced air convection drying oven for 3.5 hours at 100ºC and then reweighed (AOAC, 1990). Total nitrogen was determined for the dried milk samples using a carbon, hydrogen, and nitrogen (CHN) elemental gas analyzer (Model 2400, Perkin Elmer, Norwalk, CT). This method has been validated against the macro Kjeldahl procedure with nitrogen recovery around 98-99% and has been used at Smithsonian National Zoological Park to measure milk nitrogen for a wide variety of species. The obtained nitrogen value was multiplied by 6.38 to determine the amount of CP in the milk (Jones, 1931). Total lipid was measured using a micro modification of the Roese–Gottlieb procedure by means of sequential extractions with ethyl alchohol, diethyl ether, and petroleum ether (Hood *et al*., 2009). Total sugar was analyzed by the phenol–sulphuric acid colorimetric procedure (Dubois *et al*., 1956; Marier and Boulet, 1959) using ultraviolet spectroscopy and lactose monohydrate standards. Gross energy (GE) content of the milk was calculated as: 9.11 * fat + 3.95* sugar + 5.86 * CP (Perrin, 1958). This formula has been validated against values from adiabatic bomb calorimetry for milks from rhesus macaques (Hinde *et al*., 2009) and bongos (Petzinger *et al*., 2014). Values are expressed on a wet weight basis, both as g/g (%) and on a per energy basis (mg/kcal). The mg of nutrient per kcal of milk was calculated by: 1000 * (nutrient expressed in g/g)/GE.

Results

Milk composition did not vary over the collection period. For example, the water content ranged from 90.43–91.05%, and sugar, the next most common milk constituent, ranged from 6.31–7.22%. Mean values for water, sugar, protein, fat, and ash content are given in Table 1. Asian rhinoceros milk has a high water content with a correspondingly high sugar and low fat content. It is similar

to milk from the white rhinoceros, although it appears higher in protein, both on an absolute basis and on an energy basis. Indeed, although the percent protein of Asian rhinoceros milk is about one-third the mean value for milk from an Asian elephant with a calf at about the same age, the milk protein on an energy basis is actually higher in the Asian rhinoceros (Table 1).

Discussion

These values must be interpreted with some caution, as they represent the results from a single cow over a single lactation. However, the results were consistent over lactation and with values from samples taken from multiple white rhinoceros cows. The value for milk protein on an energy basis has been suggested to be associated with relative growth rate (Power *et al*., 2002). If this hypotheses is true for rhinoceroses, then we predict that Asian rhinoceros calves grow faster than white rhinoceros calves and even relatively faster than Asian elephant calves. The high water content of the milk might benefit the calf by providing large amounts of water for heat regulation through evaporative water loss (Tilden and Oftedal, 1997). However, it may also suggest that lactating rhinoceros cows may face a water stress challenge, which might limit their range during lactation to areas with sufficient water.

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Table 1. Mean values for macronutrients in Asian (*Rhinoceros unicornis*) and white (*Ceratotherium simum*) rhinoceros milk.

		Asian rhinoceros	**White rhinoceros[1]**	**Asian elephant[2]**
Parameter	**Unit**	***n* = 14**	**37 (ash, *n* = 36)**	***n* = 74 (ash, *n* = 45)**
GE	kcal/g	0.41	0.37	1.44
Water	%	90.66	91.48	77.4
Ash	%	0.25	0.24	0.69[3]
Sugar	%	6.98	6.86	5.00
Fat	%	0.44	0.42	11.10
Protein	%	1.53	0.98	4.10
Protein	mg/kcal	37.1	26.7	30.0

[1]Data from Petzinger *et al*. (2012) for milk samples from 3 months to one year of calf age
[2]Data from Abbondanza *et al*. (2013) for milk samples from 6 months to 1 year of calf age
[3]The sum of Ca, P, Mg, K, and Na

CLINICAL SIGNIFICANCE OF NUTRITIONAL ISSUES

Donald L. Neiffer, VMD, MHS (One Health), CVA, Dipl. ACZM

Chief Veterinarian, Smithsonian Conservation Biology Institute, National Zoological Park, 3001 Connecticut Ave NW, Washington DC 20008, USA

Abstract

Along with disease prophylaxis and risk-based quarantine protocols, comprehensive pathology and nutritional programs are cornerstones of a holistic preventive health program for zoological collections. Unfortunately, the accepted "normal" for accredited and "modern" institutions includes a scenario that regularly exists whereby the clinical veterinarian(s) also serve as the primary pathologist and nutritionist on site. Although tissues need to be sent to pathologists for final histological evaluation, all veterinarians go through the same basic training in pathology and so can usually pass as efficient prosectors and recognize many gross necropsy findings. Where nutrition is concerned, however, the level of training in veterinary schools is fairly minimal and/or is often focused on production animal operations. Even where pet nutrition is concerned, the level of knowledge gained in veterinary school does not translate well across the numerous taxa encountered by zoological veterinarians. Consequently, most clinicians are significantly outside their "comfort zone" when it comes to prescribing diets to the collection, particularly when deficiencies, excesses, and imbalances arise.

Still, even when nutritionists or nutrition-focused staff exist, clinical veterinarians play an important role in nutritional management, particularly in the area of food safety/toxicity, but also as part of a larger team charged with constant monitoring of animal body condition and health. Understandably, clinicians approach most nutritional issues from the consequence or outcome side of things, working backwards through treatment to prevention. In comparison, husbandry and nutritional staff have a default ability to be more proactive by addressing potential behaviors (e.g. aggression, stress, competition) and food type/presentation challenges (e.g. calories, vitamins, minerals, fats, browse, fiber) at the onset of exhibit design and diet creation.

Even with the best planning, not all outcomes can be predicted and invariably nutritional issues will arise. Though there may be tendency (or desire) to put the responsibility of resolution in the clinical veterinarians' court, success where active cases/issues are concerned and prevention of future cases is better served if all stakeholders are involved. In addition to the common suspects (husbandry, nutrition, and veterinary staff), point individuals from finance, public relations, administration, horticulture, and facilities may be required or appropriate in addressing a particular issue. On the other end of the spectrum, prevention, both nutrition and veterinary staff should be involved in exhibit design and collection planning to comment on potential issues related to maintenance of a particular species. This is often of paramount importance where mixed species exhibits and/or reproductive populations are concerned.

ESTIMATING APPARENT DRY MATTER DIGESTIBILITY USING DIETARY MANGANESE IN THE COMMON MARMOSET (*CALLITHRIX JACCHUS*)

Michael L. Power, PhD[1]*, Caitlin G. Arlotta, BS[1], Jessica P. Davis, BA[2], Ricki Colman, PhD[3], Suzette D. Tardif, PhD[2]

[1]Nutrition Laboratory and Conservation Ecology Center, Smithsonian Conservation Biology Institute, National Zoological Park, 3001 Connecticut Ave NW, Washington DC 20008 USA
[2]Southwest National Primate Research Center, 7620 NW Loop 410, San Antonio TX 78227 USA
[3]Wisconsin National Primate Research Center, Southwest Commuter Path, Madison WI, 53715 USA

Abstract

Captive common marmosets (*Callithrix jacchus*) display a wide range of digestive abilities linked to intestinal inflammation which can lead to chronic diarrhea, weight loss, vitamin D deficiency, and eventually marmoset wasting syndrome and/or metabolic bone disease. Animals that appear healthy can have digestive efficiency 5–10 percentage points lower than completely healthy marmosets. Detecting animals with subacute digestive difficulties is important for overall health and because variation in digestive abilities can introduce unaccounted for variation in research studies. We tested a method of estimating apparent dry matter digestibility (ADMD) using naturally occurring manganese in the diet. Animals (n = 42) were individually housed and restricted to eating their base diet with no supplemental foods. All food given was weighed and samples taken to determine the dry matter content. All uneaten food and feces was collected every day for two, four-day digestion trials for each subject to calculate dry matter intake (DMI) and fecal output. ADMD was calculated by 1 - dry weight of feces/DMI. Manganese concentration ([Mn]) of food and feces was assayed using atomic absorption spectrophotometry, and ADMD estimated by 1 – [Mn of food]/[Mn of feces]. Estimates of ADMD ranged from below 65% up to almost 85%. Estimates of ADMD by the total collection method were higher than by Mn concentration, but the two estimates were strongly correlated (r = 0.913, P < 0.001; Figure 1). Mn concentration in feces can be used to estimate the digestive ability of marmosets assuming a controlled, constant concentration in the feed.

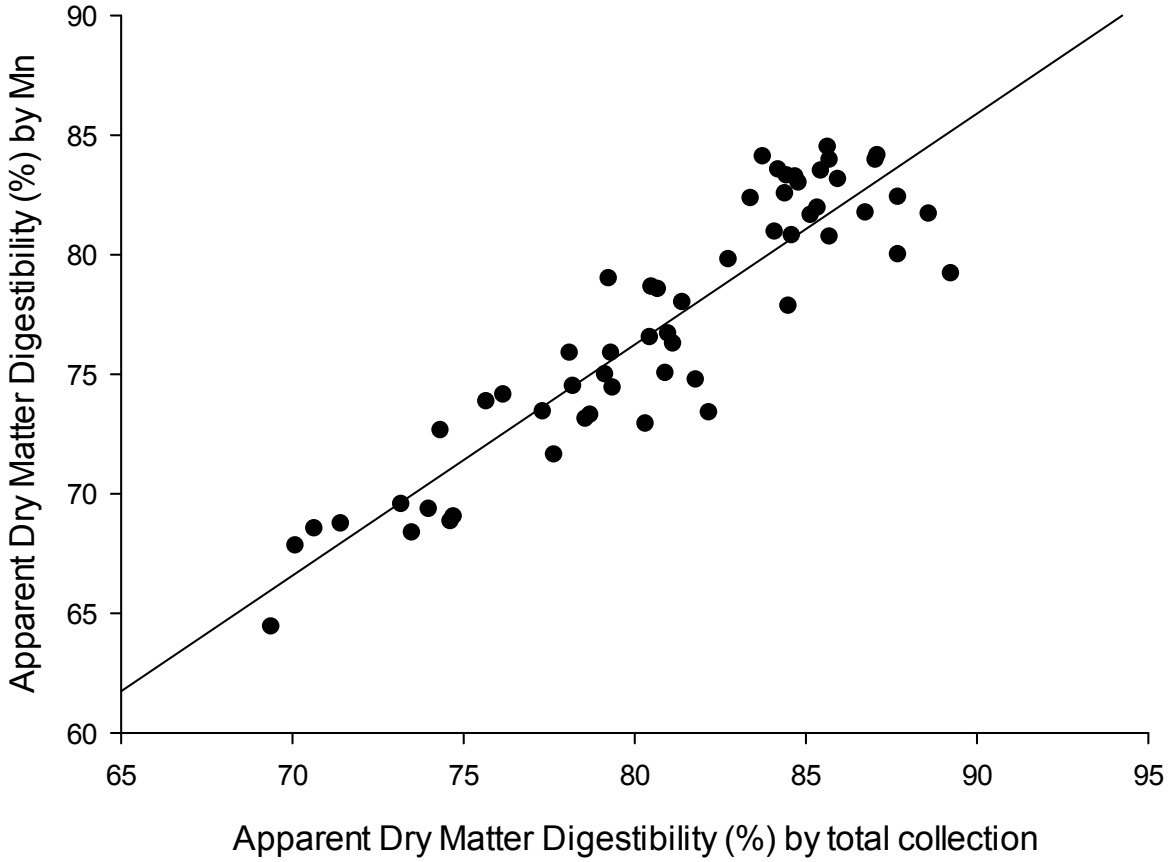

Figure 1. The estimates of apparent dry matter digestibility by fecal Mn concentration versus the total collection method.

VITAMIN D STATUS IN WILD TOQUE MACAQUES (*MACACA SINICA*) IN SRI LANKA

Michael L. Power, PhD[1]*, Wolfgang P. J. Dittu, PhD[1,2]

[1]Nutrition Laboratory and Conservation Ecology Center, Smithsonian Conservation Biology Institute, National Zoological Park, 3001 Connecticut Ave NW, Washington DC 20008 USA
[2]National Institute of Fundamental Studies, Hatana Rd., Kandy 20000, Sri Lanka

Abstract
The vitamin D receptor is found on most cells, including active immune cells, implying that vitamin D has important biological functions beyond calcium metabolism and bone health. Although captive primates should be given a dietary source of vitamin D, under free-living conditions, vitamin D is not a required nutrient but rather is produced in skin when exposed to UV-B light. The circulating level of 25-hydroxyvitamin D (25-OH-D) considered adequate for human health is a topic of controversy. Levels of circulating 25-OH-D sufficient for good health for macaques and other Old World anthropoids are assumed to be the same as human values, but data from free-living animals are scant. This study reports values for 25-OH-D and the active vitamin D metabolite, 1,25-dihydroxyvitamin D (1,25$[OH]_2$ D), for wild, forest-ranging toque macaques (*Macaca sinica*) in Sri Lanka. Plasma samples were obtained from 8 adult males, 7 juvenile males, 6 young nulliparous females, 9 adult females not pregnant or lactating, and 11 lactating adult females. Mean values for the complete sample were 61.3 ± 4.0 ng/ml for 25-OH-D and 155.6 ± 8.7 pg/ml for 1,25$[OH]_2$ D. There were no significant differences for either metabolite among age and sex classes. Values from the literature for circulating 25-OH-D in captive macaques are three times higher than those found in this wild population; however, 1,25$[OH]_2$ D values in captive animals were similar to the wild values. The data from this study indicate that anthropoid primates exposed to extensive sunlight will have circulating values of 25-OH-D generally above 30 ng/ml, providing some support for the Endocrine Society recommendations for humans. Current dietary vitamin D supplementation of captive macaques likely exceeds requirement. This may affect metabolism and immune function, with possible consequences for macaque health and biomedical research results.

Introduction
The primary known biological actions of vitamin D are in support of calcium metabolism and bone health (Pludowski *et al*., 2013). However, the vitamin D receptor is found on most cells, including active immune cells, pancreatic beta cells, bronchial epithelial cells, skin epithelial cells, testes, and mammary gland (Wang *et al*., 2012). Varying evidence indicates potential roles for vitamin D in immune function, metabolism, and cell differentiation (Rosen *et al*., 2012; Pludowski *et al*., 2013). Adequate vitamin D status appears to be beneficial to health in more ways than for calcium metabolism and bone.

There is continuing controversy over the appropriate levels of circulating 25-OH-D that represent good health for humans. Based on data regarding bone health, the Institute of Medicine (IOM) panel concluded that a circulating level above 20 ng/ml 25-OH-D is sufficient (IOM, 2011; Ross *et al*., 2011; Rosen *et al*., 2012). Other researchers have questioned this finding, citing both a concern that circulating levels of 25-OH-D at 20 ng/ml have not been shown to be sufficient for

bone health for all populations and that other non-skeletal functions of vitamin D potentially important for health, which the IOM report discounts based on inadequate evidence, may yet be shown to be important and require higher circulating levels of 25-OH-D (Heaney and Holick, 2011; Hollis and Wagner, 2013). The Endocrine Society defined deficiency as circulating 25-OH-D of less than 20 ng/ml but also defined 20–29 ng/ml as insufficiency, with a recommended level of above 30 ng/ml (Holick *et al*., 2011).

Levels of circulating 25-OH-D sufficient for good health for macaques and other Old World anthropoid primates are assumed to be the same as human values, but data from free-living animals are scant. This study reports values for 25-OH-D and the active vitamin D metabolite, 1,25-dihydroxyvitamin D (1,25$[OH]_2$ D), for wild toque macaques (*Macaca sinica*) in Sri Lanka. This paper provides partial results from the study; complete results and discussion can be found in Power and Dittus (2017).

Materials & Methods

Plasma samples were obtained from 8 adult males, 7 juvenile males, 6 young nulliparous females, 9 adult females not pregnant or lactating, and 11 lactating adult females. The research was in compliance with all legal and ethical requirements of the government of Sri Lanka and the Smithsonian Institution. All animals were individually recognized and were of known age (Dittus and Thorington, 1981). Procedures for the capture and release of macaques had been described earlier (Hoelzer *et al*., 1994). Animals were baited into live traps, anesthetized with ketamine hydrochloride (Ketalar, Parke-Davis Co.), and a blood sample was drawn. Animals were categorized by sex, age, and reproductive status into the following groups: adult male (n = 8, age range 9.2–22.4y), juvenile male (n = 6, 4.4-6.2y), nulliparous female (n = 7, 3.6–6.0y), adult female not pregnant or lactating (n = 7, 7.2–24.4y), lactating adult female (n = 13, 6.3–20.5y), or pregnant adult female (n = 4, 9.5–21.4y).

Sample collection and analyses methods

The blood was centrifuged, the plasma removed to a cryovial, placed in liquid nitrogen, and shipped to the Nutrition Laboratory of the Smithsonian National Zoological Park in Washington, DC. The plasma samples were stored at –20°C until they were shipped on dry ice to the laboratory of Dr. Michael F. Holick, Boston University School of medicine, where they were analyzed for 25-OH-D using a competitive protein binding assay as described in Chen and colleagues (1990a), and 1,25$[OH]_2$ D by the methods described in Chen and colleagues (1990b).

Statistical analyses

Concentrations of 25-OH-D (ng/ml) and 1,25$[OH]_2$ D (pg/ml) are presented as median and mean ± SEM. The relation between the two vitamin D metabolites was assessed using Pearson correlation. Differences among age-sex categories were assessed using analysis of variance. Qualitative comparisons were made with published data from free-ranging rhesus macaques (*Macaca mulatta*) on Cayo Santiago supplemented with vitamin D fortified food (Vieth *et al*., 1987) and captive macaques with minimal sunlight exposure but fed vitamin D-fortified diets (Shinka *et al*., 1983; Marx *et al*., 1989; Ziegler *et al*., 2015).

Results and Discussion

The maximum concentrations the assays could measure were 150 ng/ml for 25-OH-D and 260 pg/ml for 1,25[OH]$_2$ D. Values above those maximums were set to 151 ng/ml and 261 pg.ml for statistical purposes. The ranges of values for the concentrations of both 25-OH-D (16 to 151 ng/ml) and 1,25[OH]$_2$ D (71 to 261 pg/ml) were substantial. The mean values for the complete sample were 61.3 ± 4.0 ng/ml for 25-OH-D and, excluding the pregnant females, 149.3 ± 8.2 pg/ml for 1,25[OH]$_2$ D. There were no significant differences in serum concentrations for either metabolite among the age and sex classes nor between lactating and nonreproductive females (Table 1). The values for 25-OH-D and 1,25[OH]$_2$ D were not correlated (r = -0.187, P = 0.248).

Circulating concentrations of 25-OH-D in captive macaques fed diets with moderate levels of vitamin D (e.g. 1.5 IU/g) were similar to the values for wild toque macaques; however, values for feral (Vieth *et al*., 1987) and captive (Ziegler *et al*., 2016; Marx *et al*., 1989) macaques (*Macaca mulatta* and *M. fascicularis*) fed diets supplemented with high levels of vitamin D were two to four times higher than the values found in this wild population (Table 2). Values from the literature for circulating 1,25[OH]$_2$ D values in managed feral and captive macaques were not different from the values for the free-ranging animals from this study, regardless of the level of vitamin D supplementation (Table 2).

Conclusions

The levels of circulating 25-OH-D recommended by the Institute of Medicine (IOM, 2011) for adequate human health (above 20 ng/ml) and, by extension, for other anthropoid primates in captivity have been called too conservative and driven by a concern regarding health risks of over-supplementation and high circulating levels of 25-OH-D, a concern for which there is scant evidence and that appears implausible from an evolutionary perspective (Heaney and Holick, 2011). The data from feral (Vieth *et al*., 1987) and captive (Marx *et al*., 1989; Ziegler *et al*., 2015) macaques fed diets with high (above 6 IU/g) vitamin D appear to bear out that the risk of high supplementation may be overestimated, as these populations were both healthy and had levels of circulating 25-OH-D about 7–10 times higher than the IOM minimal level (Table 2). However, the data from this study imply that captive macaques likely are being over-supplemented with vitamin D, as their circulating 25-OH-D levels exceed the wild values by several-fold. Diets with 1.5 IU/g and 2.4 IU/g of vitamin D resulted in circulating levels of 25-OH-D that matched the values from the wild toque macaques from this study (Table 2). Although we know of no reports of concerns regarding vitamin D toxicity in captive macaques, animal care staff and veterinarians might consider whether maintaining animals at levels of circulating 25-OH-D apparently well above "natural" circulating levels is appropriate. In humans, both low (less than 30 ng/ml) and moderate-to-high circulating 25-OH-D (above 56 ng/ml) were associated with a higher risk of tuberculosis (Nielsen *et al*., 2010). Human epidemiological studies have found an association of increased health risk at high levels of circulating 25-OH-D, including all-cause mortality (Sempos *et al*., 2013), though causality has not been shown. In most cases, the increase in risk at high levels of 25-OH-D is much less than the increase in risk for levels below 30 ng/ml (Sempos *et al*., 2013), indicating that high 25-OH-D (below toxic levels) is less a health risk than very low levels. Finally, the fact that 25-OH-D levels in wild populations generally are lower than that of captive animals does not necessarily indicate that the high levels in captivity are detrimental to health and well-being in the captive environment.

The take-away messages from this study are: 1) median and mean values for circulating 25-OH-D in wild macaques under natural sunlight conditions are threefold higher than the IOM minimal levels for human health; 2) current dietary supplementation of captive macaques results in circulating 25-OH-D levels at least twofold higher than the "natural" levels; 3) this potential over-supplementation raises mild concerns regarding possible issues of vitamin D-related changes in metabolism and immune function that may affect health and/or influence the results of biomedical studies.

Acknowledgements
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Table 1. Median and mean ± standard error (SEM) for 25-OH-D and 1,25[OH]$_2$ D by different age, sex and reproductive classes for free-living toque macaques (*Macaca sinica*).

Classification	***n***		**25-OH-D (ng/ml)**	**1,25[OH]$_2$ D (pg/ml)**
Juvenile male	7	median	62	152
		mean ± SEM	71.3±8.4	177.3±17.1
Juvenile female	6	median	63	150.5
		mean ± SEM	60.0±10.0	155.7±19.6
Adult male	8	median	66	117.5
		mean ± SEM	72.1±12.8	121.0±15.7
Adult female neither pregnant nor lactating	9	median	56	150
		mean ± SEM	53.6±6.2	149.4±21.7
Lactating adult female	11	median	53	142
		mean ± SEM	55.4±7.0	148.4±15.5
All animals	41	median	60	142
		mean ± SEM	61.6±4.0	149.3±8.2

Table 2. Mean ± SEM values for 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D for captive rhesus (*Macaca mulatta*) and cynomologus (*M. fascicularis*) macaques.

Classification	***n***	**25-OH-D (ng/ml)**	**1,25[OH]$_2$ D (pg/ml)**
Rhesus macaque (Vieth *et al*., 1987)			
Juvenile males	10	165±26	201±48
Juvenile females	12	163±43	145±33
Adult males	10	218±21	125±14
Adult females	13	221±22	163±57
Rhesus macaque (Ziegler *et al*., 2015)[1]			
Adult males and females	25	155±5.5	206±19
Cynomologus macaques (Ziegler *et al*., 2015)[1]			
Adult males	25	165±6.9	193±15
Rhesus macaque (Marx *et al*., 1989)			
Adult males and females; diet with 1.5 IU/g vitamin D	3	68±8	Not measured
Diet with 6 IU/g vitamin D$_3$	6	144±10	Not measured
Cynomologus macaques (Marx *et al*., 1989)			
Adult males and females; diet with 1.5 IU/g vitamin D	3	44±3	Not measured
Diet with 6 IU/g vitamin D$_3$	6	96±6	Not measured
Rhesus macaques (Shinki *et al*., 1983)			
Adult females; diet with 2.4 IU/G vitamin D	6	50±4	100±5

[1]Diets for both macaque species in Ziegler *et al*. (2015) had 8 IU/g vitamin D

PROXIMATE COMPOSITION OF MILK OF CAPTIVE NINE-BANDED ARMADILLOS (*DASYPUS NOVEMCINCTUS*)

Michael L. Power, PhD[1]*, S. Michelle Watts, BA[2], Katie L. Murtough, MS, MPP[1,3], and Frank M. Knight, PhD[2]

[1]Nutrition Laboratory and Conservation Ecology Center, Smithsonian Conservation Biology Institute, National Zoological Park, 3001 Connecticut Ave NW, Washington DC 20008, USA
[2]Division of Sciences and Mathematics, University of the Ozarks, 415 N College Ave, Clarksville, AR 72830, USA
[3]University of Maryland College Park, College Park MD, 20742, USA

Abstract
Armadillo (*Dasypus novemcinctus*) dams have a potentially unique challenge in that their pups rapidly grow a bony carapace, suggesting a high requirement for the transfer of calcium and phosphorus from dam to pups via milk. We examined milk samples from 10 armadillo dams, samples collected at days 1–6, 14–15, 33–38, and 49–51 after birth. Water, protein, sugar, fat, ash, Ca and P content were assayed using standard methods at The Smithsonian National Zoological Park Nutrition Laboratory, and gross energy (GE) was calculated from protein, sugar and fat.

Introduction
Milk is the first and sole food of mammalian neonates. No matter what the adult diet, carnivorous, herbivorous, or omnivorous, all mammals start life as lactivores. Milk provides the nutrients for metabolism, growth, and development. The macronutrients in milk are water, fat, protein, sugar, and minerals such as calcium and phosphorus. Although these ingredients are common to all milks, the relative proportions can vary tremendously among species (Oftedal and Iverson, 1995; Skibiel *et al*., 2013). The composition of a species' milk provides insights into its evolution, ecology, patterns of growth and development, and many other aspects of life history.

The nine-banded armadillo, *Dasypus novemcinctus* (Mammalia, Xenarthra, Cingulata) is the only member of this South American superorder to expand its range into the United States. Armadillo dams have a potentially unique challenge in that their pups rapidly grow a bony carapace, suggesting a high requirement for the transfer of calcium and phosphorus from dam to pups via milk. The armadillo's carapace accounts for 16% of its total body weight, and its ossification occurs mostly after birth (Vickaryous and Hall, 2006; Anderson and Benirschke, 1966).

The goals of this study were to characterize the nutrient composition of nine-banded armadillo milk across lactation and compare its milk composition with that of other mammals. Milk samples were collected at four time points during lactation from wild-captured female armadillos that gave birth in captivity, and the samples were assayed for proximate nutrient content (water, fat, protein, sugar, ash [total minerals], and calcium and phosphorus). We predicted that armadillo milk would have high concentrations of Ca and P, and, as Ca and P in milk is bound in micelles formed by casein proteins (Holt and Carver, 2012), a high protein content as well.

Materials & Methods
Ten adult female armadillos wild-captured in northwest Arkansas during the winters of 2008 and 2009 (n = 7) and 2013 (n = 3) birthed litters in the animal quarters of the University of the Ozarks.

Nest boxes were checked morning and evening for newborn litters, which allowed for the birth date to be known within approximately +/-12 h; the day a litter was discovered was considered day 0 for days postpartum (pp).

Milk samples were collected during the first week postpartum (days 1–6), and at the ends of the second week (days 14–15), fifth week (days 33–38) and seventh week (days 49–51). Dams were anesthetized IM with ketamine/domitor cocktail in 2008, 2009, and isoflurane gas in 2013. Oxytocin was administered intravenously (0.25 ml/dose, 20 USP units/ml, repeated to effect). The milk samples were stored at -20°C until analyzed at the Smithsonian National Zoological Park (SNZP) Nutrition Laboratory in summer 2013. Milk was collected from four of the dams at all time points, from 3 time points for one dam, and two time points from another. The remaining four dams are represented by a single milk sample each collected between day 2 and 10 postpartum for a total of 25 milk samples.

Nutrient assays

All samples were assayed for water (dry matter), fat, total sugar, crude protein (CP), calcium, and phosphorus using standard methods (Hood *et al*., 2009) at the SNZP Nutrition Laboratory; gross energy (GE) was calculated. Briefly, for water determination, milk samples were aliquoted, weighed, and dried in a forced convection drying oven for 3.5-4 hours at 100°C and then reweighed (AOAC, 1990). Total nitrogen (TN) was determined through a Dumas nitrogen gas analysis procedure using a carbon, hydrogen, and nitrogen elemental gas analyzer (Model 2400, Perkin Elmer, Norwalk, CT). Total nitrogen was multiplied by 6.38 to determine CP (Jones, 1931). Crude milk fat (total nonpolar lipid) was measured by a micro-Röse-Gottlieb procedure, which involves 3 sequential extractions with diethyl ether and petroleum ether following disruption of the milk fat globules with ammonium hydroxide and ethyl alcohol (Hood *et al*., 2009). Total sugar was analyzed by the phenol–sulphuric acid colorimetric procedure (Dubois *et al*., 1956; Marier and Boulet, 1959) using lactose monohydrate standards. Replicate sugar samples were read at 490 nm with a microplate reader and accompanying software (MRX TC Revelation, Dynex Technologies, Chantilly, VA). Results were multiplied by 0.95 to correct for water of crystallization in the standard.

Ash was determined by placing dried milk samples in a muffle furnace at 550°C for 8 hours. The ash was digested in nitric acid and perchloric acid on a hot plate within a perchloric acid-rated fume hood. The resultant acid digests were diluted with distilled deionized water. Calcium was measured using atomic absorption spectrophotometry (Model 800 Perkin Elmer Analyst Flame/Furnace Atomic Absorption Spectrophotometer, Perkin Elmer Co, Waltham, MA) at 422.7 nm using a nitrous oxide flame (AOAC, 1990). Phosphorus was determined by the AOAC-Modified Gomorri colorimetric method and read with a microplate reader and accompanying software (MRX TC Revelation, Dynex Technologies, Chantilly, VA) at 450 nm (AOAC, 1990; Gomorri, 1942).

Gross energy (kcal/g milk), or GE, was calculated using the formula: GE = (9.11 kcal/g * % fat + 5.86 kcal/g * % crude protein + 3.95 kcal/g * % sugar)/100. This equation has the potential to slightly overestimate gross energy because it fails to correct for non-protein nitrogen (Perrin, 1958; Oftedal, 1984). However, it has been verified against gross energy values measured by bomb

calorimetry for milk from several species, including rhesus macaques and bongo (Hinde *et al*., 2009; Petzinger *et al*., 2014).

Statistical analysis

The composition of the 21 milk samples from the six dams with longitudinal samples was investigated using analysis of covariance, with dam as the categorical parameter and days postpartum as the covariate. The relationships among milk constituents was examined using Pearson correlation.

Results

Mean values for all milk constituents at each of the four collection time points from the 21 longitudinal samples from six dams are presented in Table 1. There was no difference among dams in the composition (protein, fat, sugar, and ash) of the longitudinal milk samples after accounting for days postpartum ($P > 0.29$ for all constituents); days postpartum was a significant factor for all these constituents ($P < 0.005$ for all constituents).

A characteristic of nine-banded armadillo milk is high concentrations of both protein and minerals (ash) throughout lactation. Protein concentration was the highest of the milk solids at all time points and ash values were higher than sugar values for the latter two time points (Table 1). Milk ash content rises consistently over lactation from about 1% of milk to above 3% by one month (Table 1). Calcium and phosphorus accounted for about half of the mineral content of the milks (Table 1). Both Ca (Figure 1) and P milk content were positively correlated with milk protein content ($r = 0.8$; $P < 0.001$ for both) and highly correlated with each other ($r = 0.99$, $P < 0.001$). The GE content of armadillo milk is relatively high (Table 1), consistent with the high protein and moderate fat content. Armadillo milk GE increased over lactation ($r = 0.759$, $P < 0.001$), increasing from 0.912 kcal/g at days 3–6 to 1.44 kcal/g at days 49–51. Protein contributes most of the energy during the first two weeks of lactation (average 52% of GE, SD = 7.5) and does not change significantly with time. Fat energy increases rapidly over the lactation period and by 35 days pp contributes a similar percentage of energy as that provided by protein (33-51 days pp - GE average: 46% from fat, SD = 4.9, average 47% from protein, SD = 4.3). Sugar contributes much less energy to milk than either protein or fat and its contribution decreases over time from an average of 12% (SD = 4.8) to 6.9% (SD = 0.94).

Discussion

Armadillo milk is unusual in the high proportion of energy from protein and its high mineral content. These two properties of armadillo milk are likely functionally connected. Armadillo pups have an unusual growth challenge in that they must grow the bony plates that serve as armadillo armor. The high mineral content of armadillo milk provides the pups the necessary minerals to grow their bony carapace. Calcium and phosphate in milk is predominantly bound within casein micelles (Holt and Carver, 2012). A milk high in calcium and phosphorous by necessity will be high in casein protein. Armadillo milk calcium content is strongly associated with milk protein content (Figure 3), suggesting a high ratio of casein proteins to whey proteins. The high protein content of armadillo milk may be required in order to transfer the appropriate amounts of calcium and phosphorus from mother to offspring via milk for the growth of the bony plates of the carapace.

The high proportion of potential metabolizable energy in armadillo milk from protein strongly suggests that armadillo pups metabolize amino acids for metabolic energy. The digestion of the casein proteins releases the calcium and phosphorous but also provides a large amount of amino acids for metabolism. Armadillo milk protein content exceeds 10% during late lactation and probably provides a substantial excess of amino acids relative to the growth requirement. Casein proteins are not balanced, containing a large proportion of proline, a non-essential amino acid and being deficient in the sulphur amino acids. The excess amino acids from casein protein digestion likely will be catabolized for metabolic energy.

A reliance on milk protein for energy may be a feature of Xenarthran lactation. There is very limited data on milk from any of the Xenarthrans, but what there is suggests that protein is higher in concentration than either fat or sugar. Giant anteater (*Myrmecophaga tridactyla*) milk is also relatively high in protein, providing a higher percentage of GE than armadillo milk (61% of the GE; M. Power, unpublished data).

Conclusions

We suggest that the evolution of armadillo bony plates required a milk relatively high in protein content. Specifically, we predict the armadillo milk protein to be high in casein proteins to carry the necessary Ca and P to form the bony plate. We also predict that armadillo pups will utilize milk protein for metabolizable energy in addition to depositing it into tissue for growth.

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Table 1. Mean values ± SEM for nine-banded armadillo (*Dasypus novemcinctus*) milk constituents at four time points across lactation.

Parameter	Pup age 3 – 6 days	Pup age 14 – 15 days	Pup age 33 – 38 days	Pup age 49 – 51 days
n	5	6	5	5
DM (%)	16.8 ± 2.2	20.0 ± 1.0	24.9 ± 1.4	24.7 ± 0.5
GE (kcal/g)	0.912 ± .142	1.105 ± 0.080	1.394 ± 0.029	1.439 ± 0.029
Protein (%)	8.0 ± 1.2	9.1 ± 0.3	11.0 ± 0.4	11.1 ± 0.4
Fat (%)	3.6 ± 0.9	5.0 ± 0.7	7.2 ± 0.1	7.6 ± 0.3
Sugar (%)	3.0 ± 0.2	3.0 ± 0.2	2.4 ± 0.1	2.3 ± 0.1
Ash (%)	1.6 ± 0.3	2.3 ± 0.2	3.4 ± 0.1	3.6 ± 0.1
Ca (%)	0.41 ± 0.12	0.70 ± 0.07	1.13 ± 0.04	1.17 ± 0.06
P (%)	0.26 ± 0.06	0.42 ± 0.04	0.62 ± 0.02	0.65 ± 0.03

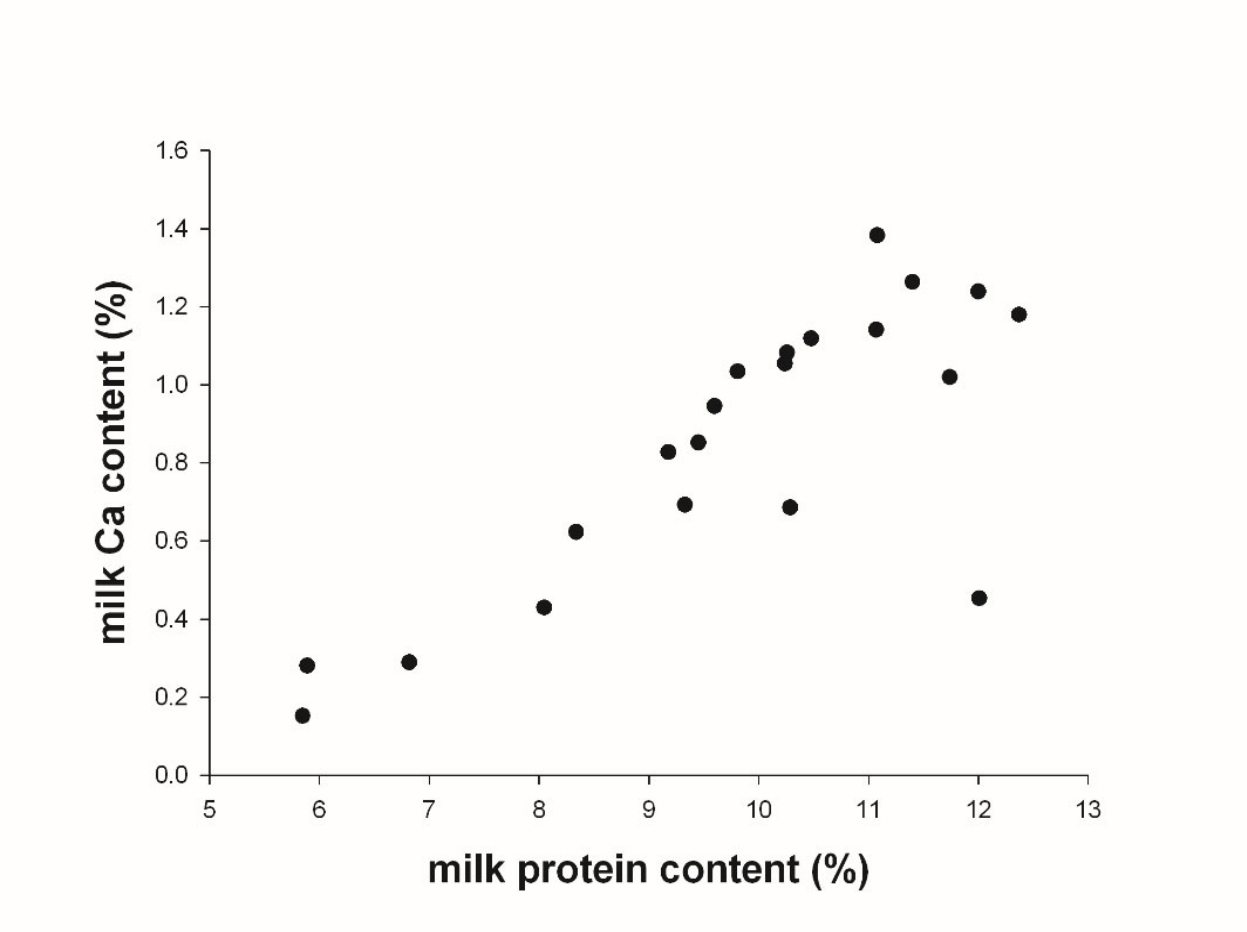

Figure 1. Nine-banded armadillo (*Dasypus novemcinctus*) milk Ca concentration is highly correlated with milk protein concentration. Values are on an As-fed basis.

EDIBLE CENTERPIECES: REDUCE, REUSE, RECYCLE

Debra A. Schmidt, PhD[1]* and Barbara Henry, MS[2]*

[1]Saint Louis Zoo, One Government Drive, St. Louis, MO 63110, USA
[2]Cincinnati Zoo & Botanical Garden, 3400 Vine Street, Cincinnati, OH 45220, USA

Abstract

The Nutrition Departments at Saint Louis Zoo and Cincinnati Zoo & Botanical Garden have partnered with their Events/Group Sales Departments to create unique produce centerpieces for zoo events. This relationship enables the animals to receive items that may be too costly for a typical zoo nutrition budget, while saving money (estimated 30-40%) over floral centerpieces, which are often thrown away after each event. These centerpieces are perfect for business lunches, semi-formal dinner parties, donor events, and themed occasions like the Cincinnati's Zoo & Botanical Garden's Farm to Zoo celebrations. The earlier a plan of what centerpieces will be used is established for the event the better; however, at least a week is necessary to secure the produce supplier. These arrangements/centerpieces are easy to make at the last minute and can be assembled the day before or on the day of the event. Event planners arrange the event, the nutritionist approves the items desired, either the planner or nutritionist can order the produce, and the arrangement can be assembled by a variety of people including the event staff, the nutritionist, an intern, or zoo volunteers. Ideas for the centerpieces are a combination of the "guests" arranging the event, staff in the events areas, and nutritionists. High quality produce vendors that are typically used by the zoo for animals or for human foods have been the suppliers utilized; some specialty items may require a more specialized vendor. Items that have been tried at these two zoos include: asparagus (variety of colors), baby bok choy, Romanesco broccoli, Swiss chard, grapes, baby pineapples, artichokes, heirloom tomatoes, radishes, Brussels sprouts, raspberries, rainbow carrots, mini sweet peppers, mini eggplant, kumquats, blueberries, coconuts, cactus pads, colored cauliflower, broccolini, pomegranates and prickly pears. Many leafy greens do not hold up well at room temperatures. The sky is the limit as to what produce items could be used. Food artists should not be ill when assembling arrangements and use the same set of standards established at that institution as staff use when preparing animal diets. Notes are inserted with the centerpieces explaining what they are and politely asking the guests attending the events to not touch them as they will be offered to the animals after the event. Arrangements should be unassembled and offered to animals the next day, if possible; some items may keep a few days longer depending on what it is. St. Louis and Cincinnati offer the food items as a substitution for like items in the diet. These items are not offered as a sole source, and special attention is paid to offering certain types of produce to certain animals following the same guidelines established in animal diets. Risk of disease transmission is determined by the individual institution. St. Louis and Cincinnati do not offer items from arrangements to primates. Additionally, St. Louis does not offer these items to elephants.

SHORT-BEAKED ECHIDNAS (*TACHYGLOSSUS ACULEATUS*): AN INSECTIVOROUS HERBIVORE

Michelle Shaw, MSc[1]*, Lydia Tong, MA VetMB[1], Phoebe Meagher, PhD[1], Kate Brandis, PhD[2], and Debashish Mazumder, PhD[3]

[1]Taronga Conservation Society Australia, Bradleys Head Road, Mosman, NSW 2088, Australia
[2]University of New South Wales, High Street, Kennington, NSW 2052, Australia
[3]Australian Nuclear Science and Technology Organisation, Locked Bag 2001 Kirrawee DC, NSW 2232, Australia

Abstract

Short-beaked echidnas (*Tachyglossus aculeatus*) are considered myrmecophages, carnivores that specialize in eating termites and ants. Dogs and cats have been used as the carnivore models to develop their artificial diets. Echidnas appear to do well on artificial diets, often living 50+ years in captivity. A recent review of necropsies of echidnas at Taronga Zoo revealed pathology in captive echidnas that may have been previously dismissed as incidental due to their longevity. Between 1908 and 2007, only 7 captive births were recorded in Australia. Only four captive-bred echidnas survived beyond 18 months of age in the American zoo population between 1903 and 2011. Nutrition may be the fundamental issue which underlies poor reproductive success. Stomach lesions seen in captive echidnas are reminiscent of ruminal acidosis gastritis which is instigated by large amounts of highly digestible carbohydrate and low fiber levels in herbivore diets. Taronga introduced a diet with lower simple sugars and added cellulose in 2014 and has since had echidnas born each year. Another Australian zoo made a similar diet change years earlier and in the last decade bred 13 echidnas. Stable isotope analysis (SIA) shows a $\delta^{15}N$ value in wild echidna quills which positions echidnas in a trophic level similar to deer. Initial results from isotopic analysis and application of diet mixing model in conjunction with stomach morphology, pathology, pH, and microbiome indicate that short-beaked echidnas are more similar to herbivores than carnivores.

AN EXAMINATION OF VARIOUS METHODS OF DIET PACKAGING

Kerri A. Slifka, MS

Nutrition Department, Dallas Zoo, 650 S. R.L. Thornton FWY, Dallas, TX 75203 USA

Abstract
Diet packaging is one of the most visible uses of resources from an operations perspective. It is specific to the individual institution; however, most utilize a combination of plastic bags and containers. Evaluation of the environmental impact of this packaging is essential as are the resources to appropriately clean and sanitize reusable materials. There is no single right answer.

Introduction

"Saving animals starts with saving habitats, and by choosing to act responsibly in our business practices, we provide support for our field conservation work around the world and our conservation education programs at home. We cannot, as an industry, be serious about saving wildlife without being serious about natural resource conservation". Wanda Evans, Chair of AZA's Green Scientific Advisory Group (2016).

Diet packaging is one of the most visible uses of resources from an operations perspective. As Green Teams in individual institutions look for ways to reduce plastic, packaging, and environmental impact, the topic of diet packaging frequently arises.

Utilization

What products are being utilized across our respective institutions? A NAGNOTES questionnaire resulted in responses from 30 institutions. Not surprisingly, packaging is specific to each institution's operation and combined a variety of options. Every institution uses containers, pans, buckets, or bins. These can be for delivery of pre-packaged individual diets, for bulk food sent to be prepared by keepers in the animal area, or for the individual diets themselves. Primary material used was plastic in various forms and thicknesses but also included metal, stainless steel, and repurposed produce boxes. Over 80% of responding zoos use plastic bags of various types in some capacity in their diet packaging. Other materials in the bag category included wax, biodegradable 'plastic,' and cloth mealworm bags. Of those that used plastic bags, the ultimate destination included discard (24%), recycle, reuse (57%), or a combination of both depending on use (19%). When it comes to cleaning, 52% wash by hand only, 28% use a dishwasher, and 20% use a combination or both. The nutrition department is the final cleaning location for 73% of institutions responding.

Institutions utilizing plastic bags would welcome a more eco-friendly option; however, cost and availability of appropriate products limit implementation. In more centralized systems, space for storing, drying, and delivery vehicle size also hinders moving from plastic bags to only containers. Heavy duty plastic containers that hold up to impacts and freezing would be welcomed.

Assessment

Plastics

Plastic bags are recyclable in many locations and can be made into new products. They are waterproof and thus great for wet diets, as well as being lightweight, and taking up less space both for storage and when filled with food. Plastic bags are cost effective overall, and the energy required to manufacture plastic bags is less than that of the same weight of paper bags. However, plastic bags are manufactured from petroleum-based, non-renewable products. All recycling plants do not accept plastic bags, and recycling compliance in the overall population was less than 10% in 2014 (EPA, 2016). Containers can be reused multiple times, and many can be recycled when useful life has been exhausted. However, washing for multiple use takes energy, water, chemicals, and staff time.

Paper /cardboard

Paper products are manufactured from a renewable resource. The majority of recycling programs accept paper products; 65% of paper and cardboard products were recycled in 2014 (EPA). However the paper manufacturing process generates more pollutants than plastic production. The recycling process is less energy efficient and takes up more space both in landfills and for storage (Florida State Department). Paper products are generally not suitable for extended use with wet items.

Alternative products

Alternative products encompass the full spectrum of compostable and biodegradable plastics. Biodegradable plastics must decompose within one year after customary disposal. They may degrade in soil or water. Compostable plastics degrade into soil conditioning material (aka compost) under a prescribed set of conditions. All compostable plastics are biodegradable, but not all biodegradable plastics are compostable. Bioplastics are manufactured from plant material instead of petroleum based products. They may or may not be biodegradable or compostable depending on the individual product. Neither compostable nor biodegradable plastics can be recycled in the traditional manner and can contaminate and disrupt the recycling stream. Compostable plastics will only degrade in a commercial composting facility where the temperatures reach appropriate levels. Product options are limited but continue to expand. Costs for alternative products can be up to 7x that of similar plastic products.

Machine vs hand washing

Stamminger (2004) found that a household dishwasher uses roughly 17% less water and 50% less energy than the average hand-washer (the appliances used average of about 4 gallons of water and about 1 kWh of electricity per load for a standard washing cycle). The automatic dishwasher used less detergent and got the dishes cleaner as well. In 2014, the Fort Worth Zoo looked at the cost of plastic bags vs containers, including cleaning costs. In that study based on 1000 staff hours per year, the commercial washer used 24,455 gal of water per year and 3120 kWh of energy per year. Machine washing adds the ability to sanitize with high temperatures, while hand washing requires a multistep method and appropriate mixing of chemicals to sanitize containers.

Conclusions

As stewards of the environment we must consider all options to create the least environmental impact in our operations. Budget and staff time play an important role in implementation of eco-

friendly products. This balancing act will continue as further options become available. Share your successes!

Acknowledgements
Thanks go out the following institutions or individuals for responding to the packaging questionnaire: Brookfield Zoo, Busch Gardens Tampa Bay, Caldwell Zoo, Cincinnati Zoo and Botanical Gardens, Columbus Zoo, Denver Zoo, Ellen Dierenfeld, Disney's Animal Kingdom, Fort Worth Zoo, Georgia Aquarium, Lincoln Park Zoo, Milwaukee County Zoo, Oklahoma City Zoo, Omaha's Henry Doorly Zoo, Oregon Zoo, Philadelphia Zoo, Potawatomi Zoo, Saint Louis Zoo, San Diego Zoo, San Diego Safari Park, Santa Barbara Zoo, Smithsonian's National Zoo, Toronto Zoo, Tulsa Zoo, Zoo Miami.

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CONTINUING ASSESSMENT OF VITAMIN ANALYSIS RELIABILITY ACROSS LABORATORIES: EXAMPLES IN WHITE AND BLACK RHINO SPECIES (*CERATOTHERIUM SIMUM* AND *DICEROS BICORNIS*)

Kathleen Sullivan, PhD[1,2]*, Amanda Ardente DVM, PhD[1,2], Scott Williams, MS[1], Shannon Livingston, MSc[1], Eduardo Valdes, PhD[1,2,3,4]

[1]Department of Animal Health, Disney's Animals, Science, and Environment. 1180 N. Savannah Circle, Bay Lake FL 32830, USA
[2]Department of Animal Sciences, PO Box 110910, University of Florida, Gainesville, FL 32611, USA
[3]University of Guelph, 50 Stone Road East Guelph, Ontario, N1G 2W1, Canada
[4]University of Central Florida, 4000 Central Florida Blvd. Orlando, FL 32816, USA

Abstract

As part of an ongoing assessment of vitamin E supplementation in white and black rhino species (2017–2018) at Disney's Animal Kingdom, the first 6 months of assessment utilized 2 laboratories (A and B) for vitamin E analysis in split serum samples, and 2 laboratories (C and D) for vitamin E analysis in split fecal samples. In both species (n = 3, each), fecal samples were taken every 2 weeks. Blood samples were taken every 2 weeks for black rhinos and every month for white rhinos. Fecal samples had similar vitamin E concentrations when compared between labs C and D (n = 45; r = 0.67; $P < 0.01$). However, there were major differences seen in serum vitamin E levels between labs A (1.3 ± 0.5 µg/mL) and B (0.7 ± 0.9 µg/mL), which had previously been utilized for a vitamin E study in elephants (Sullivan *et al*., 2016). There was no discernable correlation between the split serum samples (n = 46; r = 0.14; P = 0.36). A possible cause of the vitamin E laboratory discrepancy was that Lab B was performing extra dilutions on serum samples, as their stated minimum sample volume was actually a minimum volume requiring dilution, not for analysis, hence raising the minimum detection limit. This was a key communication lesson, as some of our collection animals are able to readily provide a slightly larger quantity of blood through routine collection procedures. Due to continuing questions, minimal clinical samples have been sent to Lab B for the immediate future. Lab A, while reliable, may not be open to high frequencies of clinical samples, so may not be a viable option to use long term. We continue to work to find a commercial laboratory that can be relied on for consistent serum vitamin analysis, as well as having the ability to do mineral analyses. This is critical for correct nutritional assessment and supplementation plans for sensitive exotic species such as rhinos.

Introduction

Assessment of the impact of nutrition on the health of exotic animals ideally includes clinical serum profiles including vitamin and mineral panels. However, when these are obtained as part of a yearly clinical assessment, trust in the laboratory values is essential. Diet changes are often made at Disney's Animal Kingdom based on values of vitamins or minerals in serum. One of the most common vitamins supplemented is vitamin E (□-tocopherol). Serum vitamin E concentrations have been reported to be lower in managed black and white rhinoceros when compared with free-ranging rhinos, irrespective of age and sex (Clauss *et al*., 2002; Dierenfeld, 1994). Only bilary excretion of vitamin E has been demonstrated as a route of exit from mammalian bodies, when vitamin E exceeds absorptive capacity, primarily through tocopherol transport protein in the liver

(Combs and McClung, 2016). Due to historical issues when studying vitamin E in the serum and feces of elephants, multiple labs were utilized in a similarly designed ongoing assessment study of vitamin E in white and black rhinoceros at Disney's Animal Kingdom (Sullivan *et al*., 2016).

Methods

White and black rhinos at Disney's Animal Kingdom (white: 1.2 adults; black: 2.1 adults) were assessed for vitamin E status in serum and fecal output as part of a 1-year study testing differential dosing. For the purposes of this abstract, the effect of dosing on serum and fecal vitamin levels will not be presented, but rather a comparison of split samples for each individual across time in terms of correlation. Two labs were utilized (referred to as Lab A and Lab B) for serum assessment of vitamin E as □-tocopherol. Blood samples were obtained in royal blue top vacutainers, left at room temperature for 1 hour, manually agitated, and spun down at 3500 rpm for 10 minutes. Serum was placed into cryovials and frozen at -80°C prior for a minimum of 24 hours. Serum samples were shipped monthly to laboratories on the same day. Feces were obtained as fresh sample ($\leq$ 30 minutes from defecation), flash frozen with liquid nitrogen on site, and maintained at -20°C until a monthly same day laboratory shipment. Two labs were utilized (referred to as Lab C and Lab D) for flash frozen fecal assessment of vitamin E as □-tocopherol. In both species, fecal samples are taken every 2 weeks, with blood samples taken every 2 weeks in black rhinos and once monthly in white rhinos. Control serum samples obtained from a large phlebotomy collection in a black rhino were kept at -80C, and at least one was sent to Lab A and B with every shipment. Comparisons were performed using Pearson's *r* and bivariate correlation comparisons in IBM SPSS Statistics 22 (Armonk, NY). Alpha was considered significant at $P < 0.05$.

Results

The samples considered for each lab comparison varied with $n = 49$ (fecal comparisons), and $n = 46$ (serum comparisons). There were 23 white rhino and 26 black rhino fecal samples split and analyzed. White rhino fecal samples had similar ranges on a dry matter basis between Lab C (465 ± 166 mg/kg vitamin E) and Lab D (316 ± 164 mg/kg vitamin E). Black rhino fecal samples had overlapping ranges on a dry matter basis between Lab C (1260 ± 407 mg/kg vitamin E) and Lab D (726 ± 453 mg/kg vitamin E). These split rhino samples were positively and significantly correlated for feces ($r = 0.67$; $P < 0.01$; Figure 1). There were 16 white rhino and 30 black rhino serum samples split and analyzed. Overall, the average serum vitamin E in µg/mL was found to be 1.3 ± 0.5 for Lab A and 0.7 ± 0.9 for Lab B. The split rhino samples were not correlated, nor significant for serum ($r = 0.14$; $P = 0.36$; Figure 2).

Discussion

The inconsistencies of high performance liquid chromatography analysis of vitamin E across laboratories may be due to differences in sample handling procedures, equipment calibration, and, of course, the possibility of human error. While citing a standard HPLC procedure, laboratories producing variable and inconsistent results, both between and within themselves is a long standing challenge (Greaves *et al*., 2014). In our small comparison, we found troubling lack of patterns in reliability in one lab. An entire month of serum samples was found to be below detection limits for Lab B but relatively high for Lab A; however, the control samples sent were consistent for Lab A only. Investigation into this inconsistency revealed that Lab B's minimum amount of serum requested was only enough to dilute out, rather than run straight, raising the detection limit. Rather than request more sample, the lab reported the values as undetectable. This one incidence led to

continued questions on reliability of this laboratory for this assay and questions on historical low values seen as well. If methodologies are not completely proprietary, they should be compared systematically moving forward. While fecal analysis appeared far more consistent across laboratories, some variation did occur, especially in the last month's analysis where temperature during shipment may have been a factor, despite both going overnight on ice. Lab D did have a delayed arrival that month, in hot temperatures, perhaps contributing to a variation. Prior to the month of May's samples results were $r = 0.89$ with $P < 0.001$. However, control fecal samples (extra doubles of at least one sample every shipment) were consistent for both labs C and D, indicating greater reliability of these labs.

Despite methodological similarities between laboratories there appear to be confounding factors inhibiting uniform reporting and standardization of vitamin assays (Greaves *et al*., 2014). Sending controls across time, no matter what medium of sample is sent, can be critical to assessment of and confidence in commercial laboratories. Standardizing this quality control procedure is a necessary investment to ensure proper diet supplementation for animal health.

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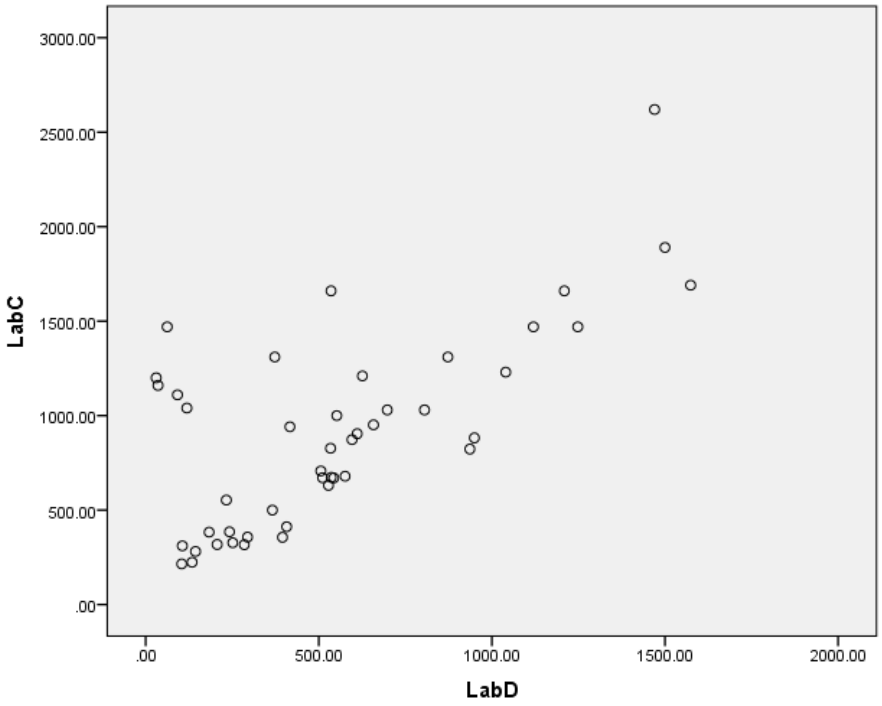

Figure 1. Comparison of two laboratories (C and D) results for vitamin E (mg/kg) in their analysis of split fecal samples for white and black rhinoceroses.

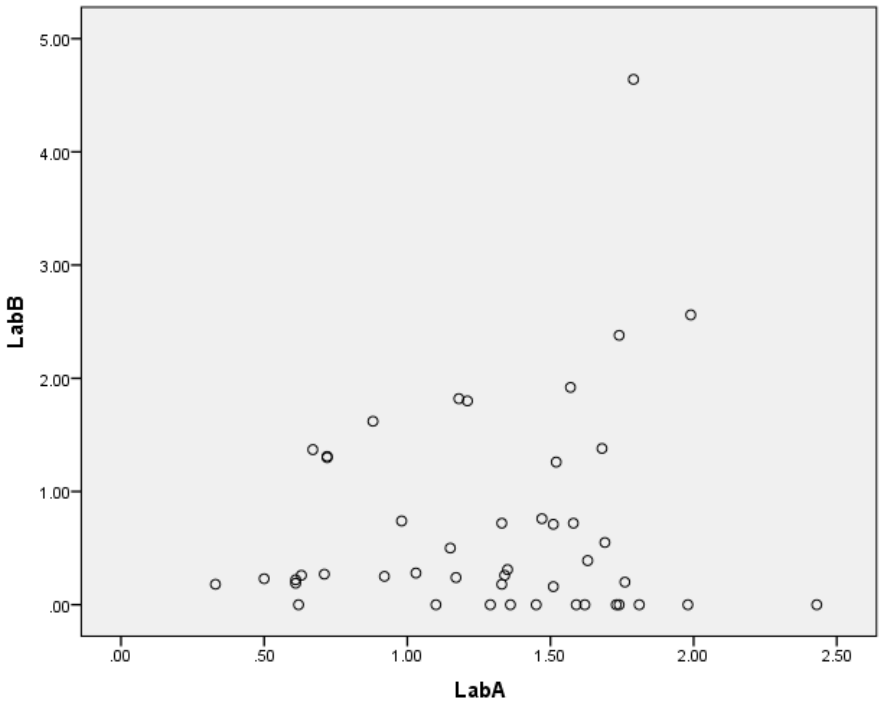

Figure 2: Comparison of two laboratories (A and B) results for vitamin E (µg/mL) in their analysis of split serum samples for white and black rhinoceroses.

SEQUENCING THE BLACK RHINO L-FERRITIN GENE: HOW ACCURATE IS OUR TESTING?

Kathleen Sullivan, PhD[1,2]*, Richard Coffey, PhD[2], Shana R. Lavin, PhD[1,2], Shannon Livingston, MSc[1], Lori K. Warren, PhD[2], Eduardo V. Valdes, PhD[1,2,3,4], Mitchell D. Knutson, PhD[2]

[1]Department of Animal Health, Disney's Animals, Science, and Environment. 1180 N. Savannah Circle, Bay Lake FL 32830, USA
[2] Department of Animal Sciences, PO Box 110910, University of Florida, Gainesville, FL 32611, USA
[3]University of Guelph, 50 Stone Road East Guelph, Ontario, N1G 2W1, Canada
[4]University of Central Florida, 4000 Central Florida Blvd. Orlando, FL 32816, USA

Abstract

Black rhinoceros (*Diceros bicornis*) under human care must be monitored for their iron status due to detrimental but poorly epidemiologically documented consequences of iron overload disorder. The recommended approach to assessing iron status in black rhinoceros examines transferrin saturation and ferritin, a species-specific protein, known to be an iron carrier as well as an acute-phase reactant. Kansas State University Diagnostic Veterinary Laboratory measures black rhinoceros ferritin by using a polyclonal rabbit anti-horse light chain (L)-ferritin antibody (Smith *et al*. 1995). While this anti-horse ferritin antibody appears to react with black rhino L-ferritin, the question remained if a black rhino-specific antibody would provide a more accurate measure. While ferritin is relatively highly conserved among species, species-specific variations in sequence may affect cross reactivity. The aim of the present study was to determine the sequence of black rhino L-ferritin and compare it to horse L-ferritin. To determine the sequence of black rhino L-ferritin cDNA, we used 5' and 3' rapid amplification of cDNA ends (RACE) of RNA extracted from 4 black rhino livers. We found that the protein encoded by black rhino L-ferritin cDNA is 90% identical to horse L-ferritin. Based on the very high degree of homology between black rhino and horse L-ferritin, it is likely that polyclonal antibodies raised against horse L-ferritin will cross-react with black rhino ferritin. Nonetheless, the black rhino ferritin sequence will now allow for the development of black rhino ferritin-specific antibodies. By establishing the most accurate available test for long-term measurement of iron load in the critically endangered black rhinoceros, preventative iron monitoring can and should be implemented as a vital component of animal management in all black rhino holding institutions.

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TECHNOLOGY USE FOR PHYSIOLOGICAL STATE: USE OF NIRS TO PREDICT PREGNANCY STATUS IN BONGO (*TRAGELAPHUS EURYCERUS)*

Kathleen Sullivan, PhD[1], Shana R. Lavin, PhD[1], Kelsey Hall, BS[1]*, Avnee Mistry[1]*, Scott Williams, MS[1], Shannon Livingston, MSc[1], Catharine J. Wheaton, PhD[1], and Eduardo V. Valdes, PhD[1,2,3,4]

[1]Department of Animal Health, Disney's Animals, Science, and Environment. 1180 N. Savannah Circle, Bay Lake FL 32830, USA
[2]Department of Animal Sciences, PO Box 110910, University of Florida, Gainesville, FL 32611, USA
[3]University of Guelph, 50 Stone Road East Guelph, Ontario, N1G 2W1, Canada
[4]University of Central Florida, 4000 Central Florida Blvd. Orlando, FL 32816, USA

Abstract
The eastern bongo (*Tragelaphus eurycerus*), a browsing African antelope species, is critically endangered. Diets under human care can be challenging for this species because of limited browse availability, and maintaining optimal health for reproduction is critical. Monitoring reproductive status in the bongo using fecal hormone analysis has not yet been explored; thus, we developed the methods for, and validated, a fecal hormone assay for detection of pregnancy in this species. As many institutions do not have an endocrinology laboratory, nor a resident endocrinologist, utilizing near infrared spectroscopy (NIRS) would be a practical tool for detecting pregnancy. This technique does not require the use of hazardous chemicals and has been applied to other species with varying success (Tolleson *et al*., 2001; Wiedower *et al*., 2012). Our objective was to develop an NIRS calibration to predict an estrogen metabolite (E1C) identified as a marker of pregnancy in dried bongo fecal samples. All animals sampled were on similar diets (Table 1). A total of 119 oven-dried fecal samples were used from 0.3 bongos to create the calibration, including two pregnancies in 0.1 bongo in the sample set (SEC = 1.04; R^2 = 0.84; cross-validation: SECV = 1.7; R^2 = 0.55). A total of 50 samples (25 pregnant and 25 non-pregnant) not included in the original calibration were used to further assess the accuracy of the prediction with moderate success. Utilizing this calibration, NIRS may be used to detect pregnancy in bongos, which has important consequences on maternal nutritional formulations and hand-rearing protocols for this species.

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The authors would like to thank dedicated members of the Science and Ituri teams for sample collection and hormone assessment. Also, thank you Dr. Amanda Ardente for all your help.

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Table 1. Average daily diet of a female bongo at Disney's Animal Kingdom.

Food Item	**Average (g/d)**
Bermuda Grass Hay	1000
Timothy Hay	500
Mazuri® Wild Herbivore Plus #5Z8W	4800
Mazuri® ZuLiFe® Wild Herbivore #5Z0X	1600
Ear Leaf Acacia	2000
Banana Leaf	500
Acacia Longfolia	1000
Romaine	582
Sweet Potato	107
Beet Pulp	100
Carrot	100
Apple	63
Supplemental Produce Enrichment*	50

*Includes green leaf lettuce, green beans, celery, turnip, bok choy, yellow squash, endive, and zucchini

CENTRALIZATION OF KNOWLEDGE FOR DISEASE UNDERSTANDING AND PREVENTION: CENTER OF EXCELLENCE FOR IRON OVERLOAD DISORDER IN BROWSING RHINOS PILOT TOOLKIT

Kathleen Sullivan, PhD[1]*, Shana Lavin, PhD[1], Mandi Schook, PhD[1], Katie Leighty, PhD[1], Shannon Livingston, MSc[1], Mark Kamhout, MS[1], Geoff Pye, DVM[1], Tammie Bettinger, PhD[1], Eduardo Valdes, PhD[1,2,3,4]

[1]Department of Animal Health, Disney's Animals, Science, and Environment. 1180 N. Savannah Circle, Bay Lake FL 32830, USA
[2]Department of Animal Sciences, PO Box 110910, University of Florida, Gainesville, FL 32611, USA
[3]University of Guelph, 50 Stone Road East Guelph, Ontario, N1G 2W1, Canada
[4]University of Central Florida, 4000 Central Florida Blvd. Orlando, FL 32816, USA

Abstract

Iron Overload Disorder (IOD) in browsing rhinos (Black and Sumatran species) is a health issue that becomes increasingly problematic over time for these species held under human care. An excess of iron beyond what is needed and contained for cell and tissue function exists in untreated browsing rhinos under human care. Excess iron damages cells and tissues throughout the body, often leading to chain reactions of destruction including liver damage and compromised immunity (Sullivan & Valdes, 2017). In black rhinos, the disorder appears to involve iron intoxication from dietary sources, rather than a genetic issue similar to human hemochromatosis, as evidenced by histology (Ganz & Nemeth, 2012; Klopfleisch & Olias, 2012; Paglia & Tsu, 2012). Regardless of the animal's husbandry, behavior, history, pedigree, and/or blood chemistry, an animal with too much iron can be considered a compromised animal and is prone to inflammation and infection. The formation of a pilot for a center of excellence for IOD in browsing rhinos was created to centralize current knowledge and continued research progress for diagnosis, prevention, and treatment options under human care. We began by reaching out to many in our zoo and research community, gathering information, current projects, and beginning a toolkit regarding practical information on management of this issue. This toolkit is built on evidence-based scientific research as well as the husbandry and medical expertise of professionals at Disney's Animal Kingdom and partner institutions. The toolkit will be a living resource, with updates made as medical discoveries are uncovered and new diagnostic and treatment methods become available. Communication across disciplines and roles can only function to better animal health in the zoo community.

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DO PINHEAD CRICKETS HAVE THE AMINO ACID PROFILE TO SUPPORT GROWTH IN LA HOTTE BUSH FROGS (*ELEUTHERODACTYLUS BAKERI)?*

Barbara Toddes BS, GC

Philadelphia Zoo, 3400 W. Girard Avenue, Philadelphia, PA 19104, USA

Abstract

The Philadelphia Zoo has been working with Haitian frogs since 2010. La Hotte bush frogs (*Eleutherodactylus bakeri*) were collected in order to develop captive husbandry/breeding protocols. *E. bakeri* was once a very common frog in southwestern Haiti, but due to habitat destruction, the species is now considered Critically Endangered (Hedges *et al.*, 2004). This species is small compared to other species of frogs typically kept at zoos. Wild adult females are approximately 4 centimeters long and may weigh up to 4 grams when gravid, while males are somewhat smaller. Females can lay communal nests with individual clutches of up to 30 or more per female (Hedges *et al.*, 1987; Hedges & Thomas, 1992; C. Martinez Rivera, Philadelphia Zoo, Philadelphia, PA, personal communication) and have a seasonal reproductive cycle from May to November. In captivity, reproduction typically begins in June, about a month after the onset of an artificial rainy season (C. Martinez Rivera, Philadelphia Zoo, Philadelphia, PA, personal communication). Froglets in this species are extremely tiny when they hatch. At this stage, springtails (*Collembola*) are offered as the only dietary item. As the froglets grow, they transition to a diet of pinhead crickets (*Acheta domestica*).

We began our breeding colony with 32 *E. bakeri*. The animals did well in captivity, and the first eggs were laid in June 2011. By September of that same year, our colony had grown to 1200 *E. bakeri*. The rate of reproduction exceeded the Zoo's expectation, and holding capacity for this species was quickly challenged. The decision was made to temporarily cease reproduction and focus on other aspects of captive management including veterinary care and husbandry. Of note and concern is that even though Zoo-hatched frogs survived to maturity and reproduced themselves, these individuals did not achieve the same adult size as founders.

In 2015, the population of frogs had dropped to a level the Zoo was comfortable with managing, and the decision was made to start breeding the animals again. The frogs were housed in the same area and under the same conditions originally used for the wild-caught (founder) animals. Subsequent to this effort to restart reproduction, the frogs produced several small clutches of eggs, and froglets emerged; however, none of the froglets survived.

The Zoo has been investigating husbandry issues that may be impacting survival and growth. The potential for nutrition to be an important or primary factor, in both the smaller size of zoo-hatched frogs and the more recent failure of froglets to survive to maturation, has been under investigation and discussion.

Specifically, we have been investigating the role of amino acids (AA): Is the AA profile affecting reproduction and the survivability of early stage froglets? Are limiting AA in the current diet impacting longer-term growth and resulting in smaller adult size?

AA are extremely important for growth. Much work has been done in livestock and laboratory animals to identify the limiting order of amino acids (from food) for growth (Anderson & Warnick, 1966; Fernandez *et al*., 1994; Panemangalore *et al.*, 1970). Although the order in which the top three dietary amino acids become limiting varies as to the one that is most limiting for a specific grain. The top three limiting AA are consistently lysine, threonine and methionine in chicks and lysine, threonine and isoleucine in rats.

To date, we have done initial analysis of pinhead crickets and have identified several important issues with prey of this size.

1. Pinhead crickets purchased from suppliers are not true pinheads - the youngest we were able to acquire were 3 day olds.
2. The first 10-day old pinhead samples we submitted to Midwest Laboratories (13611 B St. Omaha, NE 68144) were described by their analyst as non-homogeneous, since legs and other parts of the exoskeleton were apparent after grinding to the laboratory specifications. Heterogeneous mixtures have higher variation within samples.
3. The second set of 10-day old pinhead samples were collected more carefully - separating fecal and shed contaminants from insects prior to submission for analysis.

Our initial findings indicate that pinheads, as a sole food item, may be deficient in the amino acids needed to support growth.

No nutritional analysis currently exists for springtails, and that work remains to be done.

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GASTRITIS IN THE SHORT-BEAKED ECHIDNA (*TACHYGLOSSUS ACULEATUS*): PARALLELS WITH RUMINAL ACIDOSIS IN HERBIVORES AND IMPLICATIONS FOR THE UNDERSTANDING OF ECHIDNA DIGESTIVE PHYSIOLOGY

Lydia J Tong, MA, VetMB[1]*, Michelle Shaw MSc[1], Deborah Chong[2], Gabrielle Tobias BVSc, DipVetClinStud, MAppSc[1], Frances Hulst BVSc, MVS[1], Kimberly Vinette Herrin MS, DVM[1], and Larry Vogelnest BVSc, MVS, MACVSc[1]

[1]Taronga Conservation Society Australia, Bradleys Head Road, Mosman, NSW 2088, Australia
[2]University of Sydney, Faculty of Veterinary Science, Camperdown, Sydney NSW, Australia

Abstract

Short-beaked echidnas (*Tachyglossus aculeatus*) are endemic to Australia and south-eastern New Guinea (Indonesia and Papua New Guinea). In mainland Australia they feed primarily on termites. Echidnas have a single chambered stomach which is entirely lined with squamous (non-glandular) cells and is histologically analogous to the rumen. The short-beaked echidna stomach has historically been understood to function as a grinding or storage chamber for insect ingesta. Necropsy following sudden death of a captive adult echidna from a zoological collection in 2015 revealed a dramatic gastritis reminiscent of ruminal acidosis lesions common in livestock fed high carbohydrate diets. A review of gastric pathology of 20 captive and wild echidna necropsies from the Taronga Wildlife Hospital (1998-2015) revealed that 14/20 had similar gastritis lesions. One hundred percent of echidnas with microscopically normal stomachs were wild ($n = 7$), while 100% of long-term captive echidnas ($n = 8$) had moderate to marked gastritis. Five echidnas, which were wild but had been in captive rehabilitation care for a minimum of 17 days, also had gastritis. Microscopic comparison of normal and abnormal stomachs found that normal (wild) echidnas had a population of uniform coccoid bacteria lining the stomach. In contrast, the inflamed stomachs had mixed populations of rod bacteria and fungi. These anatomic, microenvironment, and pathological findings suggest that the echidna stomach may, contrary to current understanding, function like an herbivore rumen utilizing resident bacteria to assist with digestion of food. Echidna diets have historically contained large amounts of highly digestible carbohydrate – which could explain the high incidence of gastritis in captive echidnas.

RHABDOMYOLYSIS IN CAPTIVE PELICANS: CONFLICTING INDICATORS OF VITAMIN E STATUS

Kibby Treiber, PhD[1]* and Ann Ward, MS[2]

[1]Recreation and Open Spaces Department, Zoo Miami, Miami-Dade Parks, 12400 SW 152 St. Miami, FL 33177, USA
[2]Nutritional Services, Fort Worth Zoological Association, 1989 Colonial Parkway, Fort Worth, TX 76110, USA

Abstract
Captive pelicans are supplemented with vitamin E based on low levels in frozen fish diets, risk of rancidity, and requirements for other fish-eating species. Multiple incidences of captive pelican mortality have been observed (Nichols and Montali, 1987; Shivaprasad *et al*., 2002; Zollinger *et al*., 2002) associated with periods of stress and white-streaked muscles indicative of vitamin E deficiency. However, coagulopathy is another common finding in captive pelicans and is associated with vitamin E toxicity. At Fort Worth Zoo in 2012-2013 and at Zoo Miami in 2017, conflicting signs of vitamin deficiency and toxicity occurred simultaneously, as was previously published by Zollinger *et al*. (2002). Additional sampling was undertaken to better understand the vitamin E status of these pelicans. Serum values indicated normal to very high levels of circulating vitamin E (5-70 ug/mL; normal range 4-21 ug/mL; Schlegel *et al*., 2005; Ferguson *et al*., 2014) in birds prior to death. After reduction of supplementation, some brown pelicans maintained high circulating vitamin E for up to 2 years in, while other pelicans gradually returned to normal levels over several months. A number of pelicans showed spikes in circulating vitamin E during periods of stress or clinical signs despite consistent or no supplementation. Liver vitamin E values of deceased birds were normal to very high (59-705 ug/g dry tissue) compared to values for domestic chickens (45-120 ug/g dry tissue; Michigan State University Diagnostic Laboratory, 4125 Beaumont Road, MI 48910-8104) or carnivorous species (131-193 ug/g dry tissue; Ilyina *et al*., 2014). Vitamin E levels in damaged skeletal muscle were similar to very high (86-876 ug/g dry tissue) compared to values for carnivores (< 115 ug/g dry tissue; Ilyina *et al*., 2014), as were skeletal muscle levels published from pelican myopathy incidents (135-333 ug/g dry tissue; Shivaprasad *et al*., 2002; Zollinger *et al*., 2002). These findings may indicate the mobilization of vitamin E stores in response to muscle damage, possibly from stress rhabdomyolysis. Stress-related anorexia might also exacerbate mobilization of vitamin E from fat tissue stores. These findings may contraindicate vitamin E supplementation or treatment of pelicans.

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BROWSE COLLECTION AND PRESERVATION FOR WINTER IN A NORTHERN ZOO

Jennifer C. Watts, Ph.D.

Chicago Zoological Society – Brookfield Zoo, 3300 Golf Road, Brookfield, IL 60513 USA

Abstract
Zoo nutritionists agree that herbivores need browse to maintain and improve their dental, gastrointestinal, and psychological health, and ideally browse should be offered throughout the year. Unfortunately, zoos in temperate climates such as Chicago's have difficulty providing browse for their animals in the colder months, and many zoos have limited available space for browse storage. The Chiago Zoological Society – Brookfield Zoo has had a relationship with the local utility company for 6 years, wherein the utility company provides browse from trimmings around power lines. Browse from this source is vacuum sealed and placed at -20ºC for storage. Browse stored in this manner has been palatable for animals even after 8 months. This procedure may be helpful for other institutions to store browse.

Introduction

Fresh leaves, stems, and small branches ("browse") are an important part of a healthy, well-balanced diet for any herbivorous animal, and zoo nutritionists agree that more browse is needed for captive, managed species. Adding browse to the diet is intended to increase fiber content and provides both physical and psychological health benefits. Unfortunately, browse is one of the hardest feeds to provide to captive animals year-round, especially those living in seasonally variable locations such as Chicago. Most zoo herbivores only receive a fraction of the browse they would normally be eating in the wild because most, if not all, browse is completely unavailable to temperate zoos during late fall, winter, and early spring, and certain species of browse may not be available year-round even for warm-climate zoos. Many zoos have plans to collect browse during the warm months and store it for winter, but this can be limited by a lack of long-term storage space.

Nutritionally, browse is the closest food item to the natural diet of herbivores, browsers especially, as neither hay nor commercial diets can replicate the hundreds of compounds found in fresh browse. Browse consumption can improve dental health by removing tartar, improve gastrointestinal health by stimulating gut motility, and improve nutrient digestibility by activating different microbacteria that aid in digestion. Campbell *et al.* (2001) investigated the effects of offering browse to sifaka (*Propithecus spp.*) and found browse intake was around 35% of total consumption and that with increased browse, total digestible fiber and lignin consumption increased. Hatt *et al*. (2005) found that increasing browse for giraffes (*Giraffa camelopardalis*) positively affected digestible energy intake and the amount of lignin in the diet; additionally, the giraffes tended to choose browse over hay, but only when they were fed a larger amount of browse. Dierenfeld *et al*. (2006) evaluated the chemical composition and digestibility of native browse species for the Sumatran rhino (*Dicerorhinus sumatrensis*), finding that the digestibility of the native browse was higher than seen in captive species management.

The inclusion of browse in an animal's diet is enriching and improves overall welfare by providing the animal with a means to exhibit natural behaviors. Browse procurement is engaging for the animal, causing more activity, more interactivity with the exhibit, fewer stereotypic behaviors, and greater potential visitor interest in the exhibit and the species. For example, regurgitation and reingestion is abnormal in primates and can result from boredom, lack of space, or lack of control (Lukas, 1999); studies have shown that additional foraging opportunities and increased browse availability significantly decreased this behavior in great apes (Gould and Bres, 1986; Remis and Dierenfeld, 2004; Cassella *et al*., 2012; Ryan *et al*., 2012). Abnormal tongue-playing decreased in giraffes when browse was offered (Baxter and Plowman, 2001), and increased browse doubled foraging time and increased activity for elephants (*Loxodonta Africana*; Stoinski *et al*., 2000).

Materials and Methods

For the pilot test, SpaceBags™ (Ziploc®, SC Johnson) were used. portion of a maple (*Acer spp.*) branch was divided into appropriately sized pieces and placed in six bags. Two bags were placed at room temperature, 4ºC, and -20ºC and left for 12 months. After 4 months and 1 year, the leaves were visually examined and sent for protein, fiber, and minerals analysis.

An Amerivacs AVS-20 with 36" seal bar vacuum sealer was purchased from Vacuum Sealers Unlimited. Vacuum sealer bags (4 mil, 81 cm x 122 cm) were purchased from Production Packaging Equipment (Deer Park, NY) and a 75.6 L air compressor (Husky, 1.5 hp, Home Depot®2455 Paces Ferry Rd, Atlanta, GA 30339) was purchased from Home Depot.

Browse was delivered to the zoo by the local utility company, and a portion of the browse was set aside for storage. Appropriately sized branches were cut and placed in the vacuum sealer bags. Maple, mulberry (*Morus spp.*), willow (*Salix spp*), and honey locust (*Gleditsia triacanthos*) were used. After vacuum sealing, the bags were stored at -20ºC for up to 9 months.

For feeding, the bags were thawed at room temperature, then offered to the animals.

Results and Discussion

The results from the pilot study showed that the leaves stored at -20ºC maintained their color and integrity. The leaves kept at room temperature were discolored by 4 months and further discolored and drier at 1 year; they were visually unappealing. The leaves at 4ºC maintained their color and integrity at 4 months, but were discolored by 1 year. The analyses for these leaves are in Table 1. No statistics were run, as there was only 1 sample per temperature.

The browse that was frozen in the large bags were stored in the freezer in 1.22 m^3 boxes. They were removed on a scheduled basis and delivered to animal buildings. All animals consumed the thawed browse.

There is little argument that browse is important for animals on both a physiological and psychological level. Being able to store browse effectively and efficiently is beneficial for all temperate climate zoos. Freezing vacuum sealed browse seems to be an option that works.

Although there was no baseline browse to compare to, there seemed to be little difference in storage effects on nutrient concentrations measured. It must be considered though that since the

measurement of protein is based off nitrogen, and the nitrogen does not disappear over time, the protein concentrations are probably an overestimate of actual protein content. The other inorganic minerals also do not disintegrate over time, so it was expected that there wouldn't be a difference in their concentrations over time.

The browse program itself has been a great success as we have a good working relationship with the local utility company (ComEd). ComEd agrees to cut primarily from around transmissions towers to reduce the possibility of pesticides and/or pollution affecting the browse. The company has a certified arborist who oversees the tree trimmings, and he has a list of approved browse species. The arborist is on-site and chooses the areas and species to trim and also is present as the browse is off-loaded at the zoo. A twice-weekly schedule from May to October occurs at no cost to us. The ComEd team trims trees on Monday and Thursdays and delivers approximately 46 cubic meters of browse to us first thing Tuesday and Friday mornings. The volume of browse allows it to be offered daily throughout the season. Once the browse is delivered, a team of keepers load the branches onto flatbed trucks and delivers it to animal buildings throughout the zoo. The importance of providing browse is not lost on the entire animal care team in that all departments (even our Marine Mammal department) send keeper staff to help distribute it.

Additionally, the vacuum sealing and freezing of the browse has been very successful. The act of vacuum sealing is straightforward and the equipment is user-friendly. Two keepers can easily make 8–10 bags of browse in an hour. In our first full year of doing the vacuum sealing we made 170 bags. That allowed us to give out browse to eight different species every other week for the entire winter. All species which were offered the browse consumed it, this included giraffe (*Giraffa camelopardis*), okapi (*Okapia johnstoni*), colobus (*Colobus angolensis*), hyrax (*Procavia capensis*), gerenuk (*Litocranius walleri*), and orangutan (*Pongo pygmaeus*).

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Table 1. Dry matter, protein, fiber, and mineral analyses (DMB) of browse (*Acer spp.*) stored for 4 or 12 months at room temperature (RT), 4ºC, or -20ºC.

	4 months			12 months		
Nutrient	**RT**	**4ºC**	**-20ºC**	**RT**	**4ºC**	**-20ºC**
Dry matter (%)	55.1	50.1	51.2	60.3	48.9	50.1
Crude protein (%)	15.9	14.3	14.2	14.8	14.2	14.2
ADF (%)	19.4	23.6	21.8	25.1	24.2	26.3
NDF (%)	24.8	37.7	22.2	--	--	--
TDN (%)	80.4	75.7	77.7	73.9	75.0	72.6
Ca (%)	1.32	1.56	1.36	1.44	1.47	1.33
P (%)	0.36	0.30	0.31	0.32	0.32	0.31
Mg (%)	0.41	0.39	0.34	0.40	0.41	0.33
K (%)	0.93	1.00	1.09	1.16	1.04	1.17
Fe (ppm)	117	202	149	117	128	122
Cu (ppm)	16	13	13	13	16	14
Zn (ppm)	55	58	62	58	74	61
Mn (ppm)	72	74	68	75	78	70

DEVELOPMENT AND APPLICATION OF A NEW INSECT-BASED COMPLETE DIET FOR INSECTIVOROUS MAMMALS

Jennifer C. Watts, PhD

Chicago Zoological Society – Brookfield Zoo, 3300 Golf Rd., Brookfield, IL 60513

Abstract

Commercial complete diet options for insectivores are very limited, and most are an extruded diet. The extrusion process requires a high level of carbohydrates, which is not appropriate for animals that consume insects. Insects do not contain high levels of carbohydrates. They are mainly fat and protein. Chitin is an important component of insects found in the exoskeleton. It acts like a fiber source for insectivorous animals. Not having the correct fiber source can be detrimental to the gastrointestinal tract and lead to poor stool quality. This study describes the development of an insect-based complete diet and its use for mammalian insectivores. The diet was developed to meet recommendations for carnivores and adjusted based on nutrient analysis and circulating plasma concentrations of selected nutrients in animals consuming the diet. The diet is based off of four types of insects, various supplements, and agar. The resulting diet can be crumbled by hand into small "ant-sized" pieces. Palatability and consumption have been good in the species transitioned to the diet. The species include white-bellied pangolin (*Phataginus tricuspis*), echidna (*Tachyglossus aculeatus*), and giant anteater (*Myrmecophaga tridactyla*).

UPDATE ON THE PROVISION OF BROWSE AT TORONTO ZOO

Jaap Wensvoort, MSc[1]*, Elizabeth McGregor, MSc[1], Sarra Gourlie, MSc[1], and Benjamin Martin, BSc[1,2]

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

Abstract
The importance of browse in the diet programs of herbivores under human care has been well established throughout the zoological community and browse is considered good for the health and well-being of the animals. When browse is provided to browsers and intermediate browsers, they have the opportunity to express more natural foraging behaviors and have reduced unnatural behaviors. There is now reported evidence of health benefits of feeding browse to zoo animal, although it is not clear to what degree browse should be provided for nutriment, nutraceutical, and/or enrichment purposes.

In many other zoological institutions, browse is still seen as an added/occasional enrichment, and the amounts given are normally not weighed. Further, it is difficult to determine the actual edible material for the various types of browse.

Providing browse regularly throughout a zoological institution is generally a laborious task, even when it is available from designated farms, gardens, or green houses.

There are several methods currently practiced for harvesting, preparing/processing, packing, preserving, and storing browse material that require different input of staff engagement, time, and cost.

Currently, the Toronto Zoo harvests material for the provision of fresh, ensiled, and frozen browse from two off-site locations: a plantation with 10.000 dedicated willow (*Salix sp*.) and poplar (*Populus sp.)* trees/bushes, and a retired apple orchard with approximately 2,000 trees of various types. The fresh browse from the plantation is cut with saws or loppers and bundled, labelled, and weighed as accurately as practically possible. In the summer of 2016, over 10,000 kg of fresh browse was supplied. In addition, a significant (but not measured) amount of browse from variable plant species is supplied from prunings from the zoo grounds, pavilions, and/or green houses with some browse bought from third parties.

To provide browse outside of the growing season, the Toronto Zoo has established a silage production system that incorporates a mechanized and mobile hydraulic press (J. Wensvoort, Toronto Zoo) which compresses whole browse branches (15-50 cm in length and with highest diameter of approximately 2 cm) into 30 L or 120 L food-grade polyethylene barrels (Mauser Canada Ltd., Pioneer Road 1121, L7M1K5 Burlington, Canada). All filled barrels are labelled (date, plant species, and ID #), weighed, and stored outside out of direct sunlight. Barrel lids are tightly shrink-wrapped to help avoid air entry and to preserve the anaerobic state of the silage.

This browse silage is typically stored for 5-8 months before being fed; however, when there are surplus barrels, some may be fed in the next winter season at 16 or more months post-harvest without any problems. A barrel delivery schedule has been established to efficiently deliver, store, and maintain browse silage inventory, as well as to ensure that priority browsers (e.g. Giraffe (*Giraffa camelopardalis tipplskirchi*), Moose (*Alces alces*), Western lowland Gorillas (*Gorilla gorilla gorilla*), Sumatran Orangutans (*Pongo abelli*)) receive adequate amounts.

When opening the silage barrels, yeast growth is often found on the top first 5 - 10 cm of silage. This is considered harmless, but animal care staff is advised to remove and dispose of the layer of yeast growth and to feed the remaining material. Toronto Zoo recommends their staff feeding barrels within 3-5 days once opened, if ambient temperatures are between 0°C and 10°C. However, if temperatures are below freezing, the opened barrels and silage material remains safe to feed over a longer period. Keeping opened barrels in coolers is recommended.

Nutrition and animal care staff are trained to assess browse silage for quality (smell and appearance). Measureable quality controls are to test silage pH as well as fermentation end products such as lactic acid, butyric acid, etc. and by microbiologic or mycotoxin analyses. However, this is only done occasionally and outsourced to a standard Agricultural Laboratory.

Throughout the last two years, many enhancements have been made to the browse press and production system to optimize the amount harvested and improve safety. Updates include safety shields around the press arm, an emergency stop button, pressure gauge, and a folding branch stand for material breakdown by chainsaw.

The weights of packed, small (30 L) barrels range from 13-16 kg. Whereas packed, large (120 L) barrels range from 35-50 kg. Approximate totals of silage produced in 2015 and 2016 were 4,500 kg and 5,700 kg, respectively, compared to the first two years (2010 and 2011) of press use – averaging approximately 3,275 kg per year.

Browse silage can be core sampled using the specially designed core-sampler that attaches to the hydraulic arm of the press. However, this type of sampling is not the best representation of what the animal eats.

To supplement browse silage stores, browse sticks (approximately 100-150 cm in length and with diameter of 2-5 cm) are stored outside, off the ground and used for a variety of ungulate and primate species. Browse sticks (approximately 20 cm length and with a diameter between 1-5 cm) are frozen and used to supplement diets for various (mainly smaller) rodent and primate species.

While browse silage is produced as efficiently as possible using the mechanized press, labor costs and sourcing fresh plant material continue to be the main limiting factors to operational scaling at the Toronto Zoo. It is not clear how to value browse financially, and it is not clear which food or enrichment type to compare it to. Demand for browse (fresh and ensiled) is high and increasing and plans for additional dedicated browse plantations are currently being developed.

IMPACT OF REMOVING DIETARY SUPPLEMENTATION ON SERUM NUTRIENT CONCENTRATIONS IN A MANAGED POPULATION OF SOUTHERN STINGRAY (*DASYATIS AMERICANA*)

Scott M. Williams, MS[1]*, Amanda J. Ardente, DVM, PhD[1,2], Natalie D. Mylniczenko, MS, DVM, DACZM[1], Tristan L. Guttridge, PhD[3], Kathleen E. Sullivan, MS, PhD[1], Shannon Livingston, MSc[1], Eduardo V. Valdes, MSc, PhD[1,2,4,5]

[1]Department of Animal Health, Disney's Animals, Science, and Environment. 1180 N. Savannah Circle, Bay Lake FL 32830, USA
[2]Department of Animal Sciences, PO Box 110910, University of Florida, Gainesville, FL 32611, USA
[3]Bimini Biological Field Station Foundation, Miami, FL, 33176, U.S.A.
[4]University of Guelph, 50 Stone Road East Guelph, Ontario, N1G 2W1, Canada
[5]University of Central Florida, 4000 Central Florida Blvd. Orlando, FL 32816, USA

Abstract

The Walt Disney Company manages two distinct populations of female southern stingrays (*Dasyatis americana*) at The Seas®, Orlando, FL, and at Castaway Cay, Bahamas. Stingrays are housed in artificial (Orlando) or natural (Bahamas) seawater. We previously reported significant differences between these two populations based on eight years of routinely collected serum data (Williams *et al*., 2016). The aim of this study was to assess whether removal of dietary supplementation impacts serum nutrients, hypothesizing reduced managed animal serum concentrations, comparable to wild cohorts. In order to better understand our supplementation regimen, we removed daily vitamin supplementation (Mazuri® Shark/Ray-low cobalt Tablet 5M1Y, PMI Nutrition International LLC, Saint Louis, MO 63108) from the diets of The Seas® population. After 13 months, we observed reductions in total serum iodine, cobalt, copper, and manganese concentrations ($P < 0.05$) in unsupplemented rays ($n = 11$) compared to controls ($n = 36$). Wild population samples ($n = 14$, female) were collected in partnership with the Bimini Biological Field Station, Bimini, Bahamas. Compared to the wild samples, The Seas® rays without supplementation had no significant difference in serum total and inorganic iodine ($P > 0.08$, and $P > 0.5$, respectively). Although serum manganese remained numerically greater in The Seas® rays (10.6 vs 3.65 ng/kg), this was not significant ($P = 0.17$) due to high standard deviation for the sample size. Cobalt ($P < 0.001$) and copper ($P < 0.05$) both remained significantly higher in The Seas® population versus wild rays. These findings suggest the current level of recommended supplementation may not be necessary in managed southern stingrays with further research warranted to determine appropriate recommendations.

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ANALYSES OF DIET AND SERUM MINERAL CONCENTRATIONS IN AFRICAN ELEPHANTS (*LOXODONTA AFRICANA*) HOUSED AT THE NC ZOO

Jordan Wood[1]*, Elizabeth A. Koutsos, PhD[1,2], Corinne J. Kendall, PhD[3], Larry J. Minter, MS, DVM, Dipl. ACZM[3,4], Alejandra McComb, MS[5], Troy Tollefson, PhD[5], Kimberly Ange-van Heugten, PhD[1]

[1]Department of Animal Science, North Carolina State University, Raleigh, NC 27695, USA
[2]Koutsos Consulting LLC, 2084 Toad Hollow Trail, Apex, NC 27502, USA
[3]North Carolina Zoo, 4401 Zoo Pkwy, Asheboro, NC 27205, USA
[4]Department of Clinical Sciences, North Carolina State School of Veterinary Medicine, Raleigh, NC 27606, USA
[5]Mazuri® Exotic Animal Nutrition, PMI Nutrition, Land O' Lakes, Inc. 1080 C. Rd. F, MS 5380, Shoreview, MN 55126

Abstract

Elephants in human care (*Loxodonta africana*) are often over conditioned. Consequently, many zoos aim for reduced caloric intake and increased activity levels. Diets containing higher dietary browse and forage percentages with lower inclusion of pelleted components may stimulate increased foraging behavior. However, feeding increased browse with unknown nutrient profiles while decreasing pelleted nutritionally complete feed could prove problematic with respect to nutritional requirements despite stimulating wanted behaviors. The NC Zoo decided to feed a new grain-free supplement (Hay Enhancer™, Mazuri®, PMI Nutrition, Land O' Lakes, Inc. 1080 C. Rd. F, MS 5380, Shoreview, MN 55126) while increasing their daily browse offerings and eliminating the prior nutritionally complete feed. There were two goals for the current research: 1) to determine the percentage and complete mineral profile of all browse species consumed by the six NC Zoo elephants from February 2016 to January 2017, and 2) to determine if the new total diet meets recommended elephant mineral requirements by assessing both diet and circulating serum mineral concentrations. Individual elephant weights and blood samples were collected monthly. Every six weeks, a four-day complete diet weigh in and out was completed for all offered browse species, produce, and hay to analyze for nutrients. Estimated daily food intakes ranged from 61-72 kg, similar to wild African elephant consumption data. Results show 39 browse species were fed between February 2016 and January 2017 and browse consumption ranged from 5-17 kg per elephant daily, which though a minor component of the overall diet represents an increase in the percentage of dietary browse offered since 2015. Browse was analyzed for minerals (Ca, Cl, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Se, and Zn). The browse species varied dramatically in mineral content due to season and species. When complete diets were analyzed for each elephant, the intake of several minerals appear marginal or of concern within the analyzed diets. Na was deficient in both summer and winter diets (0.04 %) compared to the current recommended value (0.10 %). Cu (10.5- 10.8 ppm) and Zn (43 - 47 ppm) were marginally sufficient compared to recommended intake (10 ppm, 40 ppm) for African elephants. Fe (322 - 985 ppm) and Mn intake (50 - 68 ppm) both highly exceeded recommended intake values (50 ppm, 40 ppm). Even with the removal of the Mazuri® supplement for data comparisons, Fe and Mn remained high. These results indicate that specific browse species should be avoided for long term use within NC elephant diets due to mineral profiles inconsistent with species nutritional needs. Serum was analyzed for the minerals: Ca, Cl, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Na/K ratio, P, Se, and Zn. Serum mineral ranges for Ca,

Cl, K, Na, Na/K ratio, and Zn were within reference ranges for African elephants. Magnesium (2.2 – 3.0 mg/dL) tended towards the upper limit (0.8 - 3.0 mg/dL). Phosphorus (3.8 - 5.9 mg/dL) was above the reference range (1.07 -2.3 mg/dL) and the range for iron (59 - 188 ug/mL) often surpassed the reference range (8.4 - 151.9 ug/mL). Copper (0.77 - 1.23 ug/mL) often fell below the reference range (0.86 - 1.34 ug/mL). The data generated will enable the NC Zoo and other zoos within the region to better incorporate appropriate browse species into animal diets across seasons, and ensure that nutrient intakes are appropriate based on current knowledge.

ESTABLISHING EVOLUTIONARILY DERIVED LEVELS OF VITAMIN D THROUGH METABOLIC PROFILING IN PRIMATES

Toni Ziegler, PhD

Wisconsin National Primate Research Center, 1220 Capitol Ct, Madison, WI 53715, USA

Recent data has indicated that captive nonhuman primates have higher levels of circulating vitamin D (25-hydroxyvitamin D_3) than the naturally derived vitamin D levels found in nonhuman primates living in their native habitats. Determining the appropriate amount of vitamin D to supplement captive animals may require determining their evolutionary needs for vitamin D. Blood samples obtained from three species of baboons (*Papio*), living in their native habitat in Africa revealed different levels of circulating vitamin D. Each species had different coloration in their skin and hair. The darkest baboon, the Anubis baboon (*Papio Anubis*), had the lowest levels of circulating vitamin D. The same species in captivity in the United States had levels almost twice has high. Using a vitamin D panel, we were able to look at the metabolism of vitamin D and determine levels of vitamin D sufficiency in all three species.

SPONSOR PROFILES

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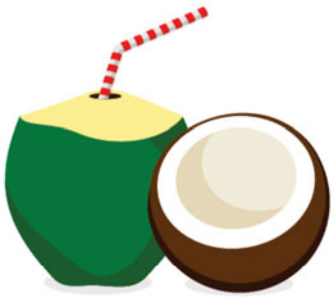
Founded in 1993 and headquartered in Evansville, Indiana, RodentPro.com specializes in the production and distribution of premium quality animal foods. We are proud to include hobbyists, commercial reptile breeding facilities, raptor sanctuaries, wildlife rehabilitators, animal rescue centers and some of the nation's largest and most respected zoos and aquariums among our broad spectrum of customers. Our company sets the industry standards for quality, value and convenience by offering unsurpassed quality, breeder direct pricing and year-round availability. As a reptile breeder, zoo/aquarium curator or bird of prey owner you know that your animals' health and longevity are directly related to proper nutrition. RodentPro.com customers can be assured that all of our animals are fed a scientifically designed and nutritionally complete diet resulting in a premium quality food item. RodentPro.com offers a 100% customer satisfaction guarantee including guaranteed frozen delivery of all products and exact sizing specifications. We also guarantee the quality, appearance, and freshness of every product we ship.



FLORIDA COCONUTS

Larry Siegel

Ian Siegel

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.

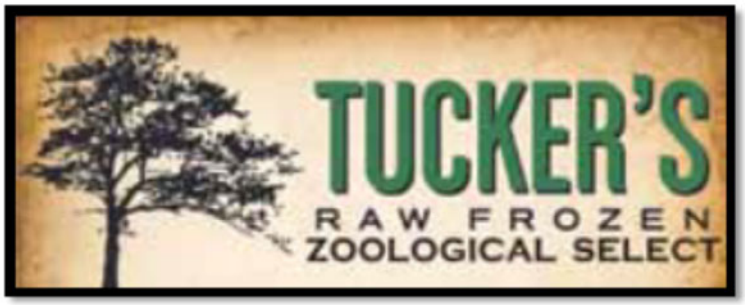
At Milliken Meat Products Ltd we are always ready to satisfy the needs of your "wild" carnivores.

Sincerely,

Jason De Rosa
Milliken Meat Product Ltd

Email: millikenmeat@hotmail.com

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



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Lynn A. Waidelich, President

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Contact Info

Zach Burton, owner
zach@burtonsbamboogarden.com
513-646-7687

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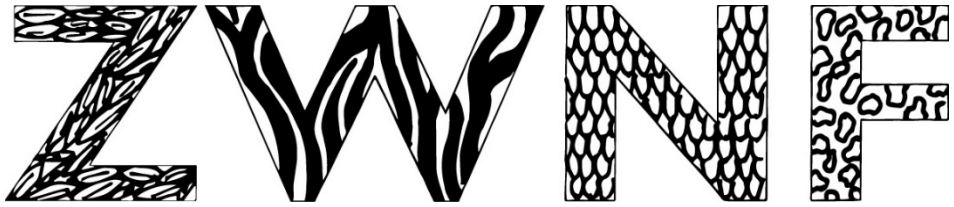

The Zoo and Wildlife Nutrition Foundation

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

Bionic Bait
95 NW 13th Ave
Pompano Beach, FL 33069
954-941-4665
866-848-9289
www.bionicbait.com

Burton's Bamboo Gardens
7352 Gheils Carroll Rd
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Marion Zoological
14149 21st Ave N
Plymouth, MN 55447
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Mazuri® Exotic Animal Nutrition
Purina Animal Nutrition LLC
1080 County Road F West, MS 5370,
Shoreview, MN 55126
800-227-8941
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McRoberts Sales Co, Inc
P.O. Box 489
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813-645-2561
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www.mcrobertssales.com

Milliken Meat Products Ltd
3447 Kennedy Rd, Unit 1
Scarborough, Ontario
Canada M1V 3P1
905-415-0060 / 416-299-9600
millikenmeat@hotmail.com

Phat Jack's
2337 Fort Worth St
Grand Prairie, Texas 75050
817-369-3524
www.phatjackfarms.com

Raw Basics
4074 S. Kinnickinnic Ave.
St. Francis, WI 53235
414-481-9900
Cell: 312-593-2880
jeff@rawbasicsusa.com

RodentPro
P.O. Box 118
Inglefield, IN 47618-9998
812-867-7598
Fax: 812-867-6058
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