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

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

Over a period of years, a decline in pelage and skin condition in adults was appreciated; several animals were diagnosed with periarticular (primarily) swellings associated with *Mycobacterim avium* sp infection; and multiple cases of hand-reared offspring succumbing to esophageal and/or gastric rupture, and/or abnormal cartilage development resulting in extensive pathologic vertebral fractures were appreciated. Review of dietary, clinical pathology and histopathologic evaluation of morbidity and mortality in hand-reared neonates was strongly suggestive of vitamin C deficiency/insufficiency disease.
Based on a working hypothesis of vitamin C-related morbidity and mortality, supplementation of hand-reared neonates with injectable sodium ascorbate, and oral supplementation of vitamin C in drinking water of juveniles, subadults and adults was initiated, with vitamin C added to drinking water based on documented giant elephant shrew daily water intake and at a level intended to meet the vitamin C requirements of guinea pigs (as a starting point). Following onset of routine vitamin C supplementation of hand-reared neonates, morbidity ascribable to putative vitamin C deficiency in this subset of giant elephant shrews ceased. In addition, improvement in skin and pelage quality of the adults was appreciated. As of this time it has not been established whether this species may have increased requirements for vitamin C more than any other mammal species; nor has it been determined whether giant elephant shrews require exogenous dietary vitamin C.

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

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

Introduction

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

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

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

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

cellulitis. The musculoskeletal system showed moderate to severe, chronic synovitis and osteoarthritis of the stifle and tarsal joints, bilaterally (Trupkiewicz, 2007).

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

Materials and Methods

Experiment 1

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

Ascorbic Acid Supplementation

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

Experiment 2

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

Ascorbic Acid Supplementation

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

Ascorbic acid measurements were conducted at initial placement of vitamin C in the water, at 4 hours, 6 hours, and 24 hours post supplementation. The temperature, light, and humidity were

measured at each time period, as well. Water was collected (10ml) from each water container at each interval in each exhibit. Samples were wrapped in aluminum foil, frozen at -40 ^{o}F, and submitted on dry ice to Midwest Laboratory for determination of the amount of vitamin C that was present.

Ascorbic Acid Analysis

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

Results

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

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

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

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

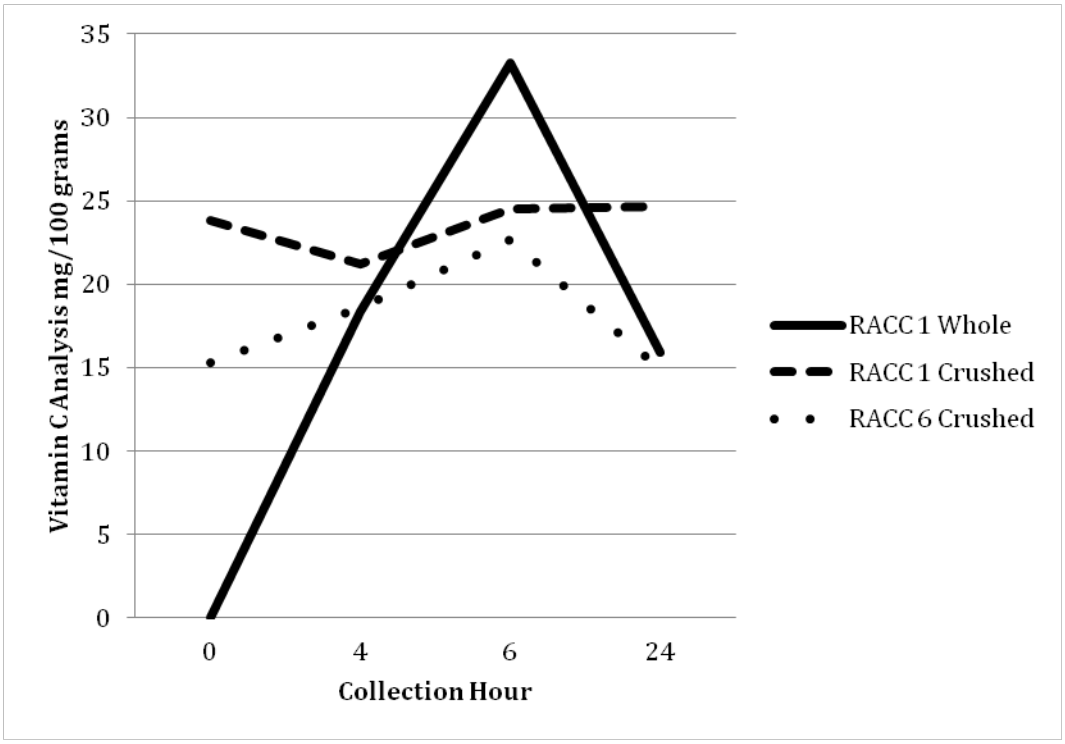

Figure 1. The concentration of vitamin C in each exhibit, either crushed half wafer or whole half wafer, over a 24 hour time period.

Discussion

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

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

Eukanuba cat food is processed in the United States and is distributed by The Iams Company, a subsidiary of Procter and Gamble, Cincinnati, OH 45202. The feed is formulated to meet the nutritional levels established by the AAFCO nutrient profile for cats at maintenance. The feed does list ascorbic acid as a feed ingredient on the label; however, the nutrient level in the feed is not listed. Since, under normal conditions, cats are able to synthesize needed ascorbic acid from glucose in the liver and high levels of ascorbic acid intake is warned against by the NRC for cats (NRC, Nutrient Requirements of Dogs and Cats 2006), the amount of ascorbic acid added to the diet through this source is believed to be minimal. Repeated managerial intake studies conducted at the Philadelphia Zoo indicate the giant elephant shrews will consume between 8 -15 grams of the cat food daily (20 grams is considered an *ad libutum* allotment for this species). The nutrient

contribution of 12 gms of cat food is used for diet formulation purposes. Giant Elephant Shrew oil is a combination of peanut oil, flaxseed oil and vitamin E liquid. The GES oil is added to the diet to increase the fat content of the diet as well as add omega-3 fatty acids and vitamin E. There is no ascorbic acid in the oil. Mealworm larvae have been reported to contain ~ 12 mg/kg Vitamin C and adult crickets 30 mg/kg as received from the commercial supplier on an as fed basis(Timberline Industries, Inc., Marion, IL) (Finke, 2002). Offered insects are routinely completely consumed by the GES. The keeper choice item can be any shrew appropriate feed item chosen by the keepers and can include fruit and vegetables as well as other insects. For the purpose of diet formulation, only the kcal added to the diet through keeper choice items are considered. The as-fed diet with no vitamin C supplementation only contains vitamin C from insects which is between 0.12 mg and 0.6 mg daily dependent on the insect chosen.

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

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

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

Conclusion

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

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