ANALYSIS OF NECTAR REPLACEMENT PRODUCTS AND A CASE OF IRON TOXICOSIS IN HUMMINGBIRDS

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Abstract

Twenty-five of 26 hummingbirds (*Trochilidae*) from 7 species previously maintained in an aviary on a commercial nectar substitute for 13 years, died over 3-month period. Gross necropsy was inconclusive; however, staining of liver and intestinal tissue showed high levels of Fe deposition. Analysis of separate bags (n=9) of the nectar product, with identical lot numbers, showed variation in Fe content from 26 to 886 mg/kg, compared to a manufacturer's specification of 40 mg/kg. A low Fe (20 mg/kg) diet was formulated for reintroduction of birds to the aviary. Samples of nectar replacement products (n=14 from 9 manufacturers) were analyzed for an array of nutrients including proximate composition, fat-soluble vitamins A and E, and minerals. Wide ranges in composition were detected among products, with no consistent variability (i.e. products with high values for one nutrient didn't necessarily have high values for any others). Crude protein ranged from 1.7 to 23.1, crude fat ranged from 1.9 to 12.8, CHO content (calculated) from 37.9 to 75.9, and ash, 0.67 to 7.0 (all % dry matter, DM, basis). Ca and P (% DM) varied 10 to 220-fold, from low concentrations (0.12 and 0.003, respectively), to highs of 1.24 and 0.67. Ca:P ratios ranged from 0.75:1 to 154:1. Vitamins A and E (IU/g) varied from lows of 0.33 and 0.005, respectively, to highs of 4,932 and 2.71, respectively. Fe content varied almost 800-fold, from 4.4 to 3,485 mg/kg DM. These analyses, in conjunction with the reported pathology, suggest a need to review Fe levels in commercial replacement products used in captive feeding programs for feeding nectarivorous species.

Introduction

There is growing awareness in the scientific and zoo cultural communities of the risk to captive wildlife, both mammals and birds, that is created by exposure to high levels of dietary iron or an environment that precludes the intake of natural regulators of iron absorption.[5,8] It has been known for several decades that certain species of birds are sensitive to moderate (250 – 500 mg/kg, dmb) dietary iron. In the 1970's, frugivorous species such as birds of paradise (*Paradiseaddae*), toucans (*Ramphastidae*), and mynahs (*Sturnidae*) were observed on necropsy to have livers marked by accumulation of iron.[3,6] A group of lories (*Chalcopsitta spp* and *Trichoglossus haematodus*) died after consuming a commercial diet containing 1,450 mg/kg iron. Classic histopathologic lesions of hemochromatosis were observerd.[10] Researchers in the United Kingdom took post mortem liver samples from birds at the Royal Zoological Society that had died during a one-year period and measured liver iron. They found that almost all specimens showed high iron content and, though there was no correlation between age at death and liver iron, there was evidence of differences between birds from different Orders (Galliformes being

lower than Passeriformes and Anseriformes). The authors noted that dietary iron intake was not excessive, though the values reported ranged from 73 mg/kg (mealworms) to 2,750 mg/kg (ground meat).[9] A review of the literature in 2002 recommended that dietary iron not exceed 100 mg/kg dmb for general avian diets, and 60 mg/kg dmb for species susceptible to iron absorption problems.[8] There have been no reports of iron storage problems in captive hummingbirds (*Trochilidae*). In November 2001, hummingbirds of various species in a walk-through aviary at the Arizona Sonora Desert Museum began dying without exhibiting symptoms of illness. This paper discusses the pathology findings from these cases and presents analytical data on several nectar replacement products collected by the Wildlife Conservation Society.

Methods

Dead birds were brought to the University of Arizona Veterinary Diagnostic Laboratory for post mortem examination. Routine necropsy and histology were performed and sections of liver were analyzed for heavy metals (lead, mercury, arsenic) and iron. Water and plant material from the aviary and the commercial nectar product were analyzed for iron content.

Samples of 14 nectar replacement products were collected from 9 manufacturers by the Wildlife Conservation Society Nutrition Laboratory, Bronx, NY, and assayed using official methods[1] for crude protein (N x 6.25), crude fat (ether extract), water-soluble carbohydrate, vitamins A and E, calcium, phosphorous, iron, copper, manganese, and zinc.

Results

By 5 months, the entire population of hummingbirds except one had died. Gross necropsy and histology of submitted birds failed to reveal the cause of death, nor was there a pattern to occasional findings of cestodiasis, ascariasis, and other incidental diseases. Screening for heavy metals was negative, but staining of liver and small intestine showed massive amounts of iron present in the cells. Retained samples of liver were analyzed for iron and found to contain from 165 to 1055 mg/kg of iron on a wet-tissue basis. Samples of plant and water sources in the aviary were collected along with insects and the commercial nectar product for iron analysis. None of the collected materials except the nectar product contained iron > 20 mg/kg. Nine nectar samples with identical lot numbers were assayed and found to contain from 26-886 mg/kg iron (dmb). The manufacturer's specification was 40 mg/kg and ferrous sulfate was listed as an ingredient. Based on these findings and the high levels of liver iron, a presumptive diagnosis of iron toxicosis was made.

The results of analyzing 14 nectar replacement products are presented in Table 1. In addition, the manufacturer's specification for a 15th product, which was not analyzed, is included for comparison. All results are reported on a dry matter (DM) basis. Since dietary energy affects feed (and therefore individual nutrient) intake, an estimate of nutrient intake per unit of Metabolizable energy (ME) was made by dividing each nutrient by the combined energy values of protein, fat and soluble carbohydrate (4.0, 9.0 and 4.0 kcal/gm, respectively) for each analyzed product. Each nutrient is listed in both its absolute value and in units per Mcal of estimated ME. There was a wide range in nutrient levels, expressed either as absolute values or on a per Mcal of ME with no apparent pattern to the variability; a product with high value for one nutrient did not

necessarily have correspondingly high values for other nutrients. Crude protein (N x 6.25) ranged from a low of 1.9% to a high of 23.1% Water-soluble carbohydrates varied from 37.9% to 75.9%. Calcium and phosphorous were equally variable, with lows of 0.12% and 0.003%, respectively, to highs of 1.24% and 0.67%, respectively.

Absolute iron content varied almost 800-fold, from 4.4 to 3485.2 mg/kg, with the magnitude of range being slightly higher when expressed as mg/Mcal ME. Average iron content for the 14 assayed products was 341.7 mg/kg (s.d. 907.1). Plotting the products in order of lowest to highest Fe content revealed a 1.5 times stair-step increase in content with the exception of the highest value which was nearly 14 times higher than the next highest value.

Discussion

The Museum successfully reared hummingbirds in this aviary for 13 years providing the commercial nectar replacement product and fruit flies (*D. melanogaster*). A decision to re-populate the aviary exhibit was made and a low nutrient diet was prepared based on work by Brice[2] who demonstrated that hummingbirds, because of a high requirement for energy, had a relatively low requirement for other nutrients per unit of energy intake. A diet with 3% protein[2] and <20 mg/kg iron was developed. Currently, the exhibit is supporting a new population with normal molting, breeding and egg laying.

Because of the lack of sufficient funds (and often animals) to perform nutritional studies, many mammal and bird diets available to zoological gardens and parks are prepared by extrapolating information gained from research on domestic animals. Besides the obvious risk of comparing the nutrient requirements of the domestic chicken or Japanese quail (both granivores) to other trophic species, there is the difference in life spans of domestic birds, grown for a set (and usually short) period of time compared to birds maintained in collections for their natural life spans. The effect of a normal 250 – 350 mg/kg iron diet may be negligible for a broiler with a life span of just under 6 weeks, but may have long-term deleterious effects on wild granivores.

The hummingbirds in the Museum aviary were maintained on a diet purported to contain 40 mg/kg iron. Over the exhibit's 13-year operation, there was what could be considered a normal attrition rate and causes of death were not confined to a single pathology. Prior to the recent deaths, liver iron was not a routine toxicological screen so no information existed on its etiologic importance. The deaths of all birds in the exhibit over the period of this study appear to have been associated with excessively high dietary iron, even though the usual histological findings of hemochromatosis (architectural and cellular derangements) were not apparent.[5] It was impossible to determine how long the birds had been exposed to the high levels since analysis for iron was not performed prior to this episode. Hummingbirds, being primarily nectarivorous, can be assumed to fall into the same group of birds that traditionally have consumed low iron diets and, therefore, may not sufficiently decrease iron absorption when body stores are adequate[3,4]. A diet with as little as 40 mg/kg of iron may in fact be high enough to cause accumulation in the liver over time and exacerbate the effects of an unusually high (>800 mg/kg dmb level. The new diet used by the Museum contains less than 20 mg/kg of iron, a somewhat arbitrary level decided upon through consensus of a task force investigating the problem. Because liver biopsy is not an option in hummingbirds due to their size, incidence of iron accumulation will be monitored post

mortem. Signs of iron deficiency (hypochromic microcytic anemia, loss of feather color, and embryonic mortality) will also be monitored.

Conclusions

Post mortem analysis of hummingbirds that died in an aviary revealed high levels of iron in the livers and intestines. Samples of the diet showed varying content of iron, from 26 to 886 mg/kg (dmb). Analysis of several commercial nectar replacement products showed extreme variability in all nutrients measured, including iron that varied from 4.4 to 3,485.2 mg/kg (dmb). These analyses, and the presumptive diagnosis of iron toxicosis in the hummingbirds, suggest that there is a need to establish guidelines for minimum and maximum iron inclusion in nectar replacement products.

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Table 1. Analysis of Nectar Replacement Products

Nutrient (DM basis)	Mean	S.D.	1	2	3	4	5	6	7
Protein, %	11.8	7.3	11.2	1.9	3.9	2.9	14.2	12.5	11.6
Crude Fat, %	5.2	3.7	9.4	1.5	1.9	3.5	4.9	4.2	2.3
Water-sol CHO, %	52.3	10.0	52.5	58.9	**65.0**	60.0	37.9	46.3	50.7
Vitamin A, IU/g	389.3	1308.9	24.2	0.4	22.2	12.5	0.3	10.1	52.9
Vitamin E, IU/kg	237.3	713.2	22.2	5.0	126.2	12.2	25.1	11.2	53.9
Calcium, %	0.46	0.4	0.6	0.3	0.2	0.2	0.1	0.3	0.3
Phosphorus, %	0.34	0.2	0.4	0.1	0.1	0.1	0.4	0.3	0.3
Ca:P Ratio	12.2	40.4	1.5	2.7	2.1	2.1	0.3	1.0	0.9
Iron, mg/kg	341.7	907.1	4.4	17.0	41.4	43.0	70.9	85.0	97.0
PER Mcal of est. ME (g prot x 4 + g fat x 9 + g sol CHO x 4)/1000									
Est. ME, Mcal/ Kg [2]	3.0	0.5	3.4	2.6	2.9	2.8	2.5	2.7	2.7
Protein, g	40.2	21.8	32.9	7.4	13.3	10.2	56.2	45.9	42.8
Crude Fat, g	16.0	9.1	27.8	5.8	6.4	12.4	19.4	15.4	8.5
Water-sol CHO, g	173.7	34.6	154.6	229.5	222.3	211.9	150.1	169.5	188.0
Vitamin A, KIU	100.4	345.5	7.1	0.2	7.6	4.4	0.1	3.7	19.6
Vitamin E, IU	67.9	187.3	6.5	1.9	43.2	4.3	9.9	4.1	20.0
Calcium, g	1.68	1.4	1.6	1.1	0.6	0.5	0.5	1.1	1.0
Phosphorous, g	1.22	0.8	1.1	0.4	0.3	0.2	1.7	1.1	1.0
Fe, mg	95.2	237.9	1.3	6.6	14.2	15.2	28.1	31.1	35.9

Table (cont.)1. Analysis of Nectar Replacement Products

Nutrient (DM basis)	8	9	10	11	12	13	14	15
Protein, %	22.7	23.1	13.0	8.5	14.9	22.0	3.5	20.0
Crude Fat, %	12.8	3.4	2.0	6.0	3.7	12.5	5.3	4.0
Water-sol CHO, %	42.8	51.9	44.0	55.5	50.5	44.0	75.9	48.0
Vitamin A, IU/g	60.6	22.5	28.7	18.9	19.5	244.8	4932.0	8.5
Vitamin E, IU/kg	34.4	44.7	27.1	80.7	32.6	135.7	2711.3	250.0
Calcium, %	0.3	0.3	0.9	1.3	1.2	0.3	0.4	1.1
Phosphorus, %	0.4	0.3	0.7	0.6	0.7	0.4	0.0	0.7
Ca:P Ratio	0.8	1.0	1.3	2.0	1.7	0.8	152.4	1.6
Iron, mg/kg	110.9	124.0	126.5	166.0	174.5	238.6	3485.2	150.0
PER Mcal of est. ME (g prot x 4 + g fat x 9 + g sol CHO x 4)/1000								
Est. ME, Mcal/ Kg [2]	3.8	3.3	2.5	3.1	2.9	3.8	3.7	3.1
Protein, g	60.2	69.9	52.7	27.3	50.5	58.4	9.7	64.9
Crude Fat, g	33.9	10.3	8.1	19.4	12.6	33.2	14.6	13.0
Water-sol CHO, g	113.5	157.0	179.0	179.1	171.2	116.9	207.5	155.8
Vitamin A, KIU	16.1	6.8	11.7	6.1	6.6	65.0	1347.9	2.8
Vitamin E, IU	9.1	13.5	11.0	26.0	11.1	36.0	741.0	81.2
Calcium, g	0.9	0.8	3.6	4.0	4.2	0.9	1.0	3.6
Phosphorous, g	1.2	0.8	2.7	2.0	2.4	1.1	0.0	2.3
Fe, mg	29.4	37.5	51.4	53.6	59.2	63.4	952.5	48.7

[a]Key to manufacturers:

1 Aves Nectar (Livefoods Direct, U.K.)
2 Quicko Nectar (Quicko Germany)
3 Nectar Mix Healesville Sanctuary (Badger Creek Rd, Healesville, VIC 3777, Australia)
4 Nektar Plus Nectar (Nekton Products, Gunter Enderle, Pforzheim, Germany)
5 Noah's Kingdom Lori Supreme (Noah's Kingdom, 31 Riordan Pl, Shrewsbury, NJ 07702)
6 Lory Life Powder (Lory Life, 768A N. Twin Oaks Valley Rd, San Marcos, CA 92069)
7 Lory Life Nectar (Lory Life, 768A N. Twin Oaks Valley Rd, San Marcos, CA 92069)
8 Nekton Lori (Nekton Products, Gunter Enderle, Pforzheim, Germany)
9 Rainbow Landing Lorikeet Nectar (Rainbow Landing, P.O. 462845, Escondido, CA 92046)
10 Wombaroo Nectar (Wombaroo, NSW, Australia)
11 Roudybush Nectar 9% Protein (Roudybush, 419 Main St., Placerville, CA 95667)
12 Roudybush Nectar 15% Protein (Roudybush, 419 Main St., Placerville, CA 95667)
13 Nekton Lori and Gelb (Nekton Products, Gunter Enderle, Pforzheim, Germany)
14 Nekton Gelb (Nekton Products, Gunter Enderle, Pforzheim, Germany)
15 Marion Lory Food. Information from manufacturer's data sheet. (Marion Zoological, 2003 E. Center Circle, Plymouth, MN 55441)