

PLASMA FAT-SOLUBLE VITAMIN AND MINERAL CONCENTRATIONS IN RELATION TO DIET IN CAPTIVE PTEROPODID BATS

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Abstract: Circulating plasma fat-soluble vitamin and mineral concentrations were compared in captive females of three species for fruit bats (*Pteropus vampyrus*, *Pteropus hypomelanus*, and *Pteropus pumilus*) fed the same diet. Daily total food intake averaged 28% of body weight on an as-fed basis or 7% on a dry matter basis. Dietary leftovers contained higher concentrations of phosphorus, magnesium, and zinc than the diet offered, suggesting some nutrient selectivity. Additionally, fecal mineral concentrations were two- to threefold higher than dietary concentrations of corresponding nutrients. Plasma concentrations of vitamin A (0.02–0.05 μg retinol/ml), vitamin D (1.50 ng 25-OH D₃/ml; 93–108 pg 1,25 diOH D₃/ml), and vitamin E (0.49–1.05 μg α -tocopherol/ml) were lower than in other herbivorous mammals, whereas plasma mineral concentrations were within normal mammalian ranges. These data may help assess the nutritional status of fruit bats.

Key words: Nutrition, Chiroptera, fruit bats, vitamin, *Pteropus* sp.

INTRODUCTION

Much of the research concerning feeding and nutrition of fruit bats has been conducted with pteropodids.^{12,20–22,29} Although food intake has been measured in some studies by examination of excreta or stomach contents of both wild^{14,18,25} and captive³⁰ bats, neither method quantifies nutrients ingested or utilized, and nutrient requirements of both megachiropteran and microchiropteran species are not well defined. Both mineral and vitamin imbalances have been described in fruit bats,^{3,11} but systematic comparisons of digestive efficiency and physiologic parameters of nutritional status have not been published.

This study was designed to investigate nutrient intake and utilization in three *Pteropus* species. Because of its relatively stable conservation status, *Pteropus hypomelanus*, the island fruit bat, has been categorized as a “husbandry research model species” by the American Zoo and Aquarium Association’s (AZA) Chiropteran Taxon Advisory Group (TAG).⁹ As such, comparative baseline data are essential for determining the suitability of this bat as a model for other pteropodid species. *Pteropus vampyrus*, the Malaysian fruit bat, and *Pteropus pumilus*, the little golden-mantled fruit bat, represent physiologic models for comparative studies as one of the largest, and one of the smallest, megachiropteran frugivores, respectively.

This study evaluated fat-soluble vitamin and mineral composition of a diet offered to and con-

sumed by a large research colony of fruit bats in comparison with recommended nutrient guidelines for frugivorous bats, documented potential differences in nutrient utilization among three species of *Pteropus*, and identified useful physiologic parameters for the assessment of nutritional status of fruit bats consuming diets of known nutrient composition. Such baseline information will contribute to our understanding of nutrition and feeding management in fruit bats and assist in evaluation and development of optimal diets for managed feeding programs globally.

MATERIALS AND METHODS

Three adult, healthy, nonpregnant female bats of each species, *P. vampyrus* (all captive bred; individuals, 869.2 \pm 46.4 g), *P. hypomelanus* (all captive bred; 492.2 \pm 19.8 g), and *P. pumilus* (two captive bred, one wild caught; 175.4 \pm 2.2 g), were selected at random from single-sex groups housed outdoors at The Lube Foundation, Gainesville, Florida 32609, USA. Prior to commencing the study, and immediately after feeding trials, each animal was weighed and physically examined to confirm health status. A blood sample (0.001% of body mass) was drawn from the ulnar vein of each bat. Bats were housed individually indoors in galvanized wire laboratory animal cages with exposure to normal photoperiod (approximately 12.5:11.5 hr light:dark) through glass windows under conditions described elsewhere.⁶

After a 1-wk period for adjustment to individual housing conditions, bats were offered their normal chopped diet (Table 1) in one afternoon meal daily for feeding during the night. Bats had been fed this

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Table 1. Composition of diets fed to three species of fruit bats (*Pteropus vampyrus*, *Pteropus hypomelanus*, *Pteropus pumilus*) at The Lubee Foundation, Gainesville, Florida. All nutrients on a dry matter basis. Data reported as mean \pm standard deviation ($n = 2$). Diets comprised (as-fed basis): 79% fruit, 16% vegetables, 5% dry commercial primate diet, and <1% additional vitamin E and calcium supplements.^a

Nutrient	Lubee Foundation diet (Mar/Apr 1995)		Chiropteran Taxon Advisory Group diet recommendation ^d
	Estimated composition ^b	Laboratory analysis ^c	
Water (%)	73.7	75.7 \pm 3.4	n.a. ^e
Protein (%)	8.5	9.5 \pm 0.6	2 to 15
Fat (%)	2.9	2.4 \pm 0.0	5 to 9
Soluble carbohydrate (%)	72.1	48.1 \pm 2.1	n.a.
Ash (%)	6.5	5.1 \pm 1.6	n.a.
Vitamin A (IU/g)	6.0 ^f	0.8 \pm 0.1 ^f	4 to 14
Vitamin D (IU/g)	0.4	0.4 ^g	0.2 to 2.0
Vitamin E (IU/kg)	125.8	40.2 \pm 14.8	11 to 56
Ca (%)	0.5	0.2 \pm 0.1	0.5 to 1.0
P (%)	0.2	0.3 \pm 0.0	0.4 to 0.9
Mg (%)	0.1	0.2 \pm 0.0	n.a.
Cu (mg/kg)	7.0	12.1 \pm 3.4	n.a.
Fe (mg/kg)	74.1	221.6 \pm 37.2	n.a.
Mn (mg/kg)	19.4	31.9 \pm 7.3	n.a.
Zn (mg/kg)	23.1	42.7 \pm 5.4	n.a.

^a Test diet composition (by fresh weight): 36% apples, 13.6% peeled bananas, 12.1% unpeeled cantelope, 7.9% grapes, 6.8% pears, 2.6% dates or figs, 9.2% steamed carrot or sweet potato, 6.6% kale, 5.2% ZuPreem Primate (Premium Nutritional Products, Inc., Mission, Kansas 66202, USA), 0.12% calcium carbonate, and <1.0% Emcelle vitamin E supplement (Stuart Products, Inc., Bedford, Texas 76021, USA).

^b Calculated from weighted nutrient values of dietary ingredients.

^c Actual nutrient values in diet quantified by laboratory analysis.

^d Fruit Bat Husbandry Manual, American Zoo and Aquarium Association Chiropteran Taxon Advisory Group, 1995.

^e n.a. = not analyzed or not available.

^f Vitamin A levels calculated without estimated contribution of carotenoids in produce.

^g Only a single sample analyzed.

quantity offered equalled approximately one-half of the animal's body weight. Feeding bowls were hung near the top of the cage to prevent fecal contamination, and water and NaCl blocks were constantly available. Two 5-day intake trials were conducted. All food offered was weighed, and remaining food (both uneaten in bowls and egested) and feces were collected, separated from any food contamination, and weighed daily for each individual. Fresh weights were recorded, and subsamples were pooled and frozen for later analysis. The combination of uneaten food in the bowls and food egested was subtracted from total food offered. Dehydration correction factors were used to determine total intake.⁶ Urine was not collected separately, thus some foods and feces may have been contaminated; urine remaining after evaporation during drying was not corrected for in subsequent calculations.

Dietary nutrient concentrations were estimated with the use of the Animal Nutritionist software (N² Computing, Silverton, Oregon 97381, USA), with

ing to most current data available. Frozen samples of diet, leftovers, ejecta, and feces were shipped on ice to the Wildlife Nutrition Laboratory, Wildlife Conservation Society, freeze-dried, and ground prior to nutrient analysis including proximate composition and water-soluble carbohydrates.⁶ Diet samples were also subjected to fat-soluble vitamin A and E analysis after general extraction and saponification.^{7,28} Vitamin D₃ was measured on a subsequent diet by a radioimmunoassay technique (M. F. Holick, pers. comm.). Calcium, copper, iron, magnesium, manganese, and zinc contents were assessed by atomic absorption spectrophotometry after dry digestion and dilution in 1% lanthanum solution¹⁹; phosphorus levels were measured by a colorimetric assay with a modification of AOAC method 995.11.²

Blood samples were separated immediately by centrifugation, and plasma was stored at -20°C until analysis. Plasma was assayed for retinol (a measure of vitamin A) and tocopherols (vitamin E) by using high-performance liquid chromatography

(Perkin-Elmer [PE], Inc., Norwalk, Connecticut 06859, USA) equipped with a 15-cm C18 reversed-phase column was used for separation; α -, γ -, and δ -tocopherols were monitored with a fluorescence detector (PE Model LS-1). Retinol was assessed at 325 nm with a PE Model LC-95 spectrophotometer; peak areas were analyzed against national standards (Hoffmann-LaRoche, Inc., Nutley, New Jersey 07110, USA). Levels of 25-OH calciferol and 1,25 diOH calciferol (vitamin D isomers) were measured by radioimmunoassay (Amersham Life Science, Arlington Heights, Illinois 61312, USA) at the University of Florida, Alachua. Macro- and trace mineral analyses were conducted on plasma samples via inductively coupled plasma atomic emission spectroscopy.²⁷

Pre- and posttrial plasma vitamin and mineral concentrations were compared by paired *t*-tests. No differences were noted; hence, data were pooled for subsequent species comparisons. Differences among species' means were determined by ANOVA, and patterns of differences among means were assessed by posthoc multiple comparisons tests (Tukey LSD at $P = 0.05$)²⁴ computed with SystatTM.³¹

RESULTS

Fat-soluble vitamin and mineral concentrations determined in the diet are found in Table 1, along with calculated nutrient values as well as the AZA Chiropteran TAG recommended dietary nutrient levels for fruit bats. Although the vitamin D content of the diet was as estimated, vitamins A and E and Ca concentrations were lower, and trace mineral levels were considerably higher, than calculated from ingredients.

Mean daily dry matter intakes as a proportion of body weight were $6.9 \pm 1.5\%$ for *P. vampyrus*, $6.6 \pm 0.7\%$ for *P. hypomelanus*, and $7.2 \pm 1.3\%$ for *P. pumilus*, or about 28% of body weight on an as-fed basis. Animals maintained a stable weight ($\leq 5\%$ variation) over the trial period. Food remained at the end of each day, therefore intake was not limited by the amount of diet provided. Dietary leftovers (Table 2) contained significantly higher concentrations of P, Mg, and Zn than the diet offered (Table 1), suggesting some nutrient selectivity and/or possible urinary or environmental contamination. No difference was seen in the mineral contents of ejecta samples compared with dietary leftovers, either within or among species, suggesting no specialized mechanisms for extracting minerals (or added minerals from saliva during mastication).

No significant difference in fecal mineral com-

Table 2. Composition of dietary mineral fractions remaining for three fruit bat species fed an identical diet at The Lubee Foundation, Gainesville, Florida. All nutrients on a dry basis, sample size = 6 for all values. Mean \pm standard deviation are reported; no significant differences were detected between nonconsumed fractions.

Nutrient	<i>Pteropus vampyrus</i>			<i>Pteropus hypomelanus</i>			<i>Pteropus pumilus</i>		
	Leftovers	Ejecta	Leftovers	Ejecta	Leftovers	Ejecta			
(%)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0			
(%)	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.0	0.3 ± 0.1			
(%)	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.3 ± 0.1			
(mg/kg)	10.0 ± 1.1	9.8 ± 0.2	9.0 ± 1.2	8.9 ± 0.4	8.3 ± 0.5	8.4 ± 0.9			
(mg/kg)	188.8 ± 68.0	165.5 ± 6.0	216.2 ± 41.0	154.9 ± 11.1	230.8 ± 82.5	160.2 ± 19.5			
(mg/kg)	28.4 ± 3.8	29.9 ± 3.1	34.5 ± 5.1	28.4 ± 3.9	28.4 ± 1.1	24.2 ± 3.6			
(mg/kg)	148.31 ± 6.0	139.8 ± 55.4	138.2 ± 92.9	201.4 ± 40.0	90.3 ± 8.5	175.4 ± 56.2			

Table 3. Fecal mineral composition in three species of fruit bats fed an identical diet at The Lubee Foundation, Gainesville, Florida. All nutrient values (except water) on a dry basis; mean \pm standard deviation ($n = 6$).

Nutrient	Feces composition		
	<i>Pteropus vampyrus</i>	<i>Pteropus hypomelanus</i>	<i>Pteropus pumilus</i>
Water (%)	78.5 \pm 8.0	79.0 \pm 2.5	75.0 \pm 1.8
Ash (%)	8.3 \pm 1.1	7.8 \pm 0.8	8.8 \pm 0.5
Ca (%)	0.7 \pm 0.1	0.7 \pm 0.1	0.6 \pm 0.1
P (%)	0.7 \pm 0.0	0.7 \pm 0.0	0.8 \pm 0.1
Mg (%)	0.6 \pm 0.0	0.6 \pm 0.0	0.6 \pm 0.0
Cu (mg/kg)	27.3 \pm 1.9	28.0 \pm 2.0	25.9 \pm 3.8
Fe (mg/kg)	450.8 \pm 37.1	465.6 \pm 44.9	522.9 \pm 45.0
Mn (mg/kg)	79.4 \pm 2.2	83.4 \pm 5.1	80.6 \pm 11.2
Zn (mg/kg)	1,205.1 \pm 148.6	1,641.4 \pm 175.3	1,007.2 \pm 406.2

this study, although *P. hypomelanus* tended toward higher Zn ($P = 0.06$) excretion. Fecal mineral concentrations were two- to threefold higher than dietary concentrations of corresponding nutrients; very high concentrations of Zn in feces of all bats compared with diet samples suggest possible contamination with Zn, perhaps from wire mesh flooring.

No γ - or δ -tocopherol isomer was detected in any of the plasma samples. Both plasma retinol and α -tocopherol concentrations were higher for *P. pumilus* compared with larger bats (Table 4), as were concentrations of Mn and P. No significant difference was noted between *P. vampyrus* and *P. hypomelanus* in vitamin D isomers; inadequate sample quantity prevented determination of this nutrient from *P. pumilus*. Because diets consumed by the three species were identical and were digested to the same extent,⁶ these limited data suggest species differences in nutrient metabolism.

DISCUSSION

Although dietary requirements of *Pteropus* for most nutrients remain unknown, the diet fed in this trial supports reproduction and growth of several species of frugivorous bats housed at The Lubee Foundation, including the three species studied here.¹³ Further, no significant species difference was noted in digestibility of major constituents of this diet among these three species.⁶ However, chemical analysis of the diet versus that calculated from ingredients (Table 1) revealed some potential nutrient imbalances with health implications. In particular, dietary vitamin E was only 30% of estimated concentration, and Fe, about threefold higher. It is possible that deterioration of vitamin E occurred between diet sampling and assay of this nutrient. Diet samples were frozen immediately after preparation, remained frozen until assay, and contained low lev-

els during freezer storage and thawing (as documented for other feeds⁸) is unlikely.

Vitamin E deficiency, however, has been documented in bats housed at The Lubee Foundation,¹¹ and excessive iron may precipitate oxidative damage (leading to vitamin E deficiency⁸) as well as induce hemochromatosis.³ Plasma levels of vitamin E (Table 4) measured in these bats were higher than those found in bats with vitamin E-associated cardiomyopathies.¹¹ Concentrations were, nonetheless, marginal to low compared with estimated values for other herbivorous species (1.0–3.0 $\mu\text{g/ml}$).⁵

Vitamin E concentration is low in most fruits,²³ whereas dark green plants are an important source of this nutrient in nature.⁴ The relative contribution of vitamin E from various dietary sources has not been documented in diets of free-ranging fruit bats and may provide some useful guidelines for designing appropriate diets for captive management. Although the analyzed vitamin E content of the diet used in this study (40.2 IU/kg) was within ranges suggested as adequate in other mammalian species (15–100 IU/kg),¹ excess iron could contribute to the low circulating concentrations of vitamin E measured. Additional dietary supplementation with vitamin E,¹¹ inclusion of more foliage in the diet,¹⁵ or lowering dietary iron concentration may improve vitamin E status in fruit bats.

Excess levels of other fat-soluble vitamins (A and D) interfere with dietary absorption of vitamin E in other species.^{8,10} Compared with other species for which data exist, this diet does not appear to contain an excessive amount of preformed vitamin A, and oxidation of vitamin A, which may have occurred between diet sampling and assay, is unlikely due to reasons documented previously. Fruit bats in nature would likely consume only precursors of vitamin A (carotenoids) and synthesize re-

Table 4. Plasma concentrations of nutrients in three species of *Pteropus* fruit bats fed a diet of known nutrient composition (mean \pm standard deviation, range in parentheses, $n = 6$ per species except where noted).^a

Nutrient	<i>P. vampyrus</i>	<i>P. hypomelanus</i>	<i>P. pumilus</i>
Vitamin A			
Retinol ($\mu\text{g/ml}$)	0.04 \pm 0.01 ^{bc} (0.02–0.05)	0.02 \pm 0.01 ^c (0.01–0.05)	0.05 \pm 0.01 ^b (0.03–0.05)
Vitamin D			
1,25 diOH (pg/ml)	108.0 \pm 61.1 (62.4–191.6) ($n = 4$)	93.3 \pm 62.4 (19.8–184.4) ($n = 5$)	n.a. ^d
25-OH (ng/ml)	1.51 \pm 0.65 (0.56–2.01)	1.50 \pm 0.53 (0.78–2.05)	n.a.
Vitamin E			
α -tocopherol ($\mu\text{g/ml}$)	0.49 \pm 0.21 ^b (0.28–0.84)	0.56 \pm 0.23 ^b (0.23–0.88)	1.05 \pm 0.46 ^c (0.54–1.71)
Minerals ($\mu\text{g/ml}$)			
Calcium	94.4 \pm 2.6 (90.4–97.1)	90.5 \pm 3.8 (83.6–94.8)	92.2 \pm 6.5 (85.5–103.0)
Copper	1.55 \pm 0.19 (1.35–1.81)	1.56 \pm 0.17 (1.36–1.67)	1.63 \pm 0.14 (1.50–1.71)
Iron	4.73 \pm 3.53 (1.51–9.50)	2.72 \pm 0.58 (1.99–3.66)	1.94 \pm 0.57 (1.15–2.77)
Magnesium	21.6 \pm 2.5 (18.3–24.1)	22.2 \pm 1.7 (20.1–24.4)	23.4 \pm 2.2 (20.7–27.1)
Manganese	0.04 \pm 0.0 ^b	0.05 \pm 0.01 ^b (0.04–0.06)	0.07 \pm 0.01 ^c (0.05–0.08)
Phosphorus	80.3 \pm 11.7 ^b (62.0–98.0)	86.3 \pm 7.9 ^b (75.0–95.0)	102.7 \pm 10.0 ^c (92.0–106.0)
Potassium	162 \pm 16 (145–186)	165 \pm 17 (155–199)	162 \pm 14 (146–181)
Sodium	3,333 \pm 148 (3,080–3,490)	3,290 \pm 86 (3,190–3,410)	3,460 \pm 10 (3,230–3,600)
Zinc	2.95 \pm 0.75 (2.14–4.27)	4.55 \pm 1.96 (1.87–7.30)	3.11 \pm 0.82 (2.16–4.54)

^a Data presented are pooled for pre- and posttrial samples, as noted in manuscript.

^{b,c} Means with different superscripts in rows differ significantly ($P < 0.05$).

^d n.a. = not analyzed.

synthetic abilities have been determined in fruit bats. The likely contribution of carotenoids to vitamin A status in fruit bats is unknown at this time. Carotenoid conversion to active vitamin A has not been shown to cause toxicity in any species, thus it may be presumed physiologically “safer” compared with adding higher levels of preformed vitamin A to diet mixtures for species that do not naturally encounter the nutrient in this form. Low concentrations of circulating retinol (Table 4) may be normal for bats, but comparative values are lacking. Most herbivores have retinol values ranging from 0.2 to 0.6 $\mu\text{g/ml}$.¹⁷ Clearly, this is an area warranting further research.

Dietary vitamin D was not analyzed in this study

Lubee Foundation was analyzed at a later date and found to be at an expected level. Nonetheless, prior to this trial, bats were exposed to natural sunlight outdoors, which would presumably provide adequate vitamin D synthesis, possible storage, and mobilizable reserves throughout the trial period. Recent data obtained from a fruit-eating phyllostomid suggest that 25-OH D₃ levels are normally low (<10 ng/ml)¹⁴ and can be elevated for an extended period of time with dietary supplementation. Excessive levels of dietary vitamins A or D were not evident in these bats.

The Ca:P ratio of 0.92:1 measured in our diet was marginal or low. Bats appeared normocalcemic from plasma assessment, although they may have

son with historic medical records for the facility (The Lubee Foundation, unpubl.). Additionally, analyzed dietary Cu and Zn concentrations appeared elevated compared with calculated dietary levels, but plasma concentrations of these nutrients were normal relative to other mammals and fruit bats.^{11,16}

Phosphorus and Mg concentrations were consistently higher in diet samples not consumed compared with that offered to the bats, perhaps because of contributions from saliva (although the mineral composition of bat saliva was not measured) or urine contamination and/or because of selectivity against these nutrients. No visual observation suggesting active selection against dietary ingredients high in P was recorded, but because of the marginal Ca to P ratio measured, it might be in the animals' best interest to avoid high-P dietary ingredients if possible.

Zinc in all leftover diet samples was extremely elevated compared with the diet prepared, suggesting possible contamination from galvanized caging, feeding pans, or, possibly, the plastic sheeting used for collections. Fecal mineral concentration was approximately double relative to diet samples (either offered or leftovers) for all nutrients analyzed except Zn, where fecal concentration was very high. Chewing on galvanized caging and/or ingestion of water in contact with enclosures may have contributed to these elevated fecal levels of Zn. Although no data presented here suggest any overt problem with Zn nutrition in fruit bats, dietary mineral interactions have not been investigated in detail in pteropodids, and Zn levels should be investigated in suspected cases of mineral imbalance.

Although small sample numbers may have contributed to a lack of statistical significance in this study, *P. hypomelanus* may be a suitable model for other medium to large body sized *Pteropus* species in nutrition studies. Its stable conservation status and lack of significant differences among parameters measured justify its use as a research model, particularly for highly endangered species. In general, plasma fat-soluble vitamin levels in fruit bats were lower than expected, whereas plasma mineral concentrations were within known ranges of other mammalian herbivores.

Acknowledgments: We thank the keepers at The Lubee Foundation for bat care, Dr. Darryl Heard for obtaining blood samples, Marianne Fitzpatrick for coordinating laboratory analyses, and Drs. Timothy Gross and Michael Holick for the vitamin D assays. Comments from M. Delorme, T. H. Kunz,

in revising the manuscript. This article is Publication no. 58 of The Lubee Foundation, Inc.

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Received for publication 04 June 1999

