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Plasma Tocopherol, Retinol, and Carotenoid Concentrations in Free-Ranging Humboldt Penguins (*Spheniscus humboldti*) in Chile

Roberta S. Wallace, J. Andrew Teare, Edward Diebold, Margaret Michaels, and Mary Jo Willis

Milwaukee County Zoo, Milwaukee, Wisconsin

Plasma retinol and α-tocopherol concentrations were measured in heparinized blood samples collected from 51 free-ranging adult Humboldt penguins (*Spheniscus humboldti*) residing at two colonies off the Chilean coast. Thirty samples were collected in April 1992 from penguins inhabiting the Ex-islote de los Pájaros Niños in Algarrobo, Chile. In September 1992, 21 samples were collected from birds inhabiting Isla de Cachagua, Chile. Samples were assayed for retinol, retinyl palmitate, α-tocopherol, γ-tocopherol, lutein, β-cryptoxanthin, lycopene, α-carotene, and β-carotene. Retinol, α-tocopherol, and lutein were detected in all samples, while lycopene and γ-tocopherol were not detected in any. A significantly higher percentage of samples had detectable levels of retinyl palmitate and α-carotene in April ($P < 0.001$); for β-cryptoxanthin the percentage was higher in September ($P < 0.001$). Plasma concentrations of α-tocopherol and lutein were higher in September. Alpha-tocopherol concentrations were 1,877.1 ± 99.0 (SEM) μg/dl in April compared to 2,289 ± 122.3 μg/dl in September ($P < 0.05$); lutein concentrations were 4.16 ± 0.43 μg/dl in April vs. 10.68 ± 1.02 μg/dl in September ($P < 0.001$). Retinol concentrations were not significantly different (117 ± 8.0 μg/dl in April vs. 105.3 ± 7.6 μg/dl in September). Both physiologic changes associated with season, and the change in locale may have contributed to the differences seen in the assay means and the number of samples with detectable levels.

Key words: vitamin E, vitamin A, nutrition, marine birds

INTRODUCTION

The Humboldt penguin (*Spheniscus humboldti*) is native to the Peruvian and Chilean coast and is one of the world's most endangered penguin species. In the wild,

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Address reprint requests to Dr. Roberta S. Wallace, Milwaukee County Zoo, 10001 W. Bluemound Road, Milwaukee, WI 52336.

they once numbered in the hundreds of thousands, but are now thought to number approximately 10,000–12,000 [Boersma et al., 1992]. Accidental drowning in fishing nets, habitat degradation, predation by humans for food, and environmental factors such as El Niño continue to put severe pressure on the wild population [Hays, 1986; Araya and Todd, 1988; Araya, personal communication]. Continued existence of this species may depend on successful long-term captive propagation; currently it is the only species of penguin in North American zoos and aquaria managed under an Association of Zoos and Aquaria (AZA) Species Survival Plan.

Numerous problems face institutions exhibiting picivorous birds, however. The diet consumed by a species in the wild is often unknown, and if known it may be unavailable commercially [Gailey-Phipps and Sladden, 1982]. The species of fish fed may be limited in variety, nutritionally inadequate, or unable to meet the changing physiological needs of the animal (e.g., breeding, molt). Fish are often caught seasonally and bought in large quantities, resulting in extended periods of frozen storage. The concentrations of essential nutrients, particularly vitamins (e.g., vitamin A, vitamin E, and the B vitamins), vary both within and between species of fish, and may decrease with time, even in frozen fish. In addition, the methods used to thaw fish prior to feeding may cause further deterioration in the level of vitamins [Geraci and St. Aubin, 1980; Dierenfeld et al., 1991; Wallace et al., 1992].

Both vitamin A (measured as retinol) and vitamin E (calculated from tocopherol) are necessary for normal health and reproduction in birds. Deficiencies of either vitamin can lead to poor fertility, early embryonic death, poor hatchability, suppressed immune function, and high chick mortality, even when adults are only marginally deficient and do not show clinical signs of hypovitaminosis [Jones and Hunt, 1983; West et al., 1992; Sklan et al., 1994]. Dietary vitamin supplementation is often used to compensate for poor fish quality, yet it must be done carefully; fat-soluble vitamins such as vitamins A and E are potentially toxic and oversupplementation may lead to illness and death [Bernard et al., 1989; Nichols et al., 1989]. The amount of supplementation has been largely empirical, usually based on the requirements for domestic poultry. Analysis of serum or plasma vitamin A and E levels has been performed for many species [Dierenfeld, 1989; Schweigert et al., 1991] and has been used as a measure of the adequacy of dietary intake [Dierenfeld et al., 1988; Mainka et al., 1992; West et al., 1992]. Plasma levels may vary considerably among species [Schweigert et al., 1991]; thus, when drawing conclusions about an animal's vitamin status in captivity, values should be compared to those obtained from healthy, free-ranging conspecifics.

Plasma retinol and tocopherol values have been reported for several species of free-ranging penguins [Ghebremeskel and Williams, 1988, 1989; Ghebremeskel et al., 1991], but published data were not found for wild Humboldt penguins. The aim of this project was to determine plasma tocopherol and retinol concentrations in free-ranging Humboldt penguins.

METHODS

Blood samples were collected from a total of 51 free-ranging Humboldt penguins inhabiting the Chilean coastline. The age and sex of the birds sampled were unknown, but all birds had adult plumage. None of the birds was in molt, and all appeared healthy. Individuals were marked with a water-resistant color marker to help

insure that they were not sampled more than once. Birds were caught while in their burrows, and manually restrained for blood collection. Thirty samples were collected over 4 days in April 1992 (the fall breeding cycle) from birds inhabiting the Ex-islote de los Pájaros Niños in Algarrobo, Chile. Fourteen of the birds sampled were incubating eggs. Twenty-one samples were collected over 4 days in September 1992 (the spring breeding cycle) from birds inhabiting Isla de Cachagua, Chile. At this time large numbers of birds were migrating ashore and digging fresh burrows in preparation for breeding.

Blood samples were collected from the medial metatarsal vein in 43 birds, and from the jugular vein in eight birds. Blood samples were immediately placed in tubes containing sodium heparin. The whole blood was centrifuged (3,500 RPM for 10 min) and the plasma was immediately removed, placed into storage vials (Nalgene cryovials), and stored in liquid nitrogen. The samples were transported to the US, and within 30 days of collection were sent to the University of Illinois–Chicago, Department of Nutrition and Medical Dietetics, for analysis. The methods for analysis have previously been described [Stacewicz-Sapuntzakis et al., 1987; Natta et al., 1988]. Minimum detection levels for the assays are: retinol, 0.9 μg/dl; retinyl palmitate, 0.7 μg/dl; α-tocopherol, 12 μg/dl; γ-tocopherol, 10 μg/dl; lutein, 0.3 μg/dl; β-cryptoxanthin, 0.6 μg/dl; lycopene, 1 μg/dl; α-carotene, 0.8 μg/dl; and β-carotene, 0.9 μg/dl.

The mean and standard error of the mean (SEM) for retinol, α-tocopherol, and lutein were calculated separately for each of the two study groups. The unpaired Student's *t* test was used to determine if there were statistically significant differences between the means of the two sample groups. For those assays where only one or two samples had undetectable levels (β-cryptoxanthin and β-carotene levels found in September), the mean was calculated using a value of one-half the minimum detectable level for those undetected values. Means were not calculated for assays with undetectable levels in a large number of samples. A χ^2 analysis was used to determine if there were significant differences between April and September in the number of samples with detectable levels.

RESULTS

Table 1 summarizes the results of samples collected in April and September, 1992. For those assays where results were below the minimum detectable level in a large number of samples, only the range of values is presented. Detectable levels of retinol, α-tocopherol, and lutein were found in all samples. There was no significant difference in the means of the two sample groups for retinol, but significant differences between the groups were found for α-tocopherol ($P < 0.05$) and lutein ($P < 0.001$), with higher means occurring in September. Significant differences in the number of samples with detectable levels were found for retinyl palmitate ($P < 0.001$), β-cryptoxanthin ($P < 0.001$), and α-carotene ($P < 0.001$). More samples had detectable levels of retinyl palmitate and α-carotene in April, while more samples had detectable levels of β-cryptoxanthin in September. Detectable levels of α-carotene were not found in any sample collected in September. There was no significant difference in the number of samples with detectable levels of β-carotene. Detectable levels of lycopene and γ-tocopherol were not found in any sample.

TABLE 1. Retinol, retinyl ester, tocopherol, and carotenoid concentrations found in free-ranging Humboldt penguins (*Spheniscus humboldti*) in April and September

April (n = 30) Test	# Detected/# samples	x̄ SEM	Observed range	Units
Retinol	30/30	117.0 ± 8.0	35.2–204.9	μg/dl
Retinyl palmitate	30/30	7.36 ± 0.65	1.9–16.4	μg/dl
α-tocopherol	30/30	1,877.1 ± 99.0	515–2716	μg/dl
Lutein	30/30	4.16 ± 0.43	0.6–10.9	μg/dl
β-cryptoxanthin	4/30	— —	<0.6–3.1	μg/dl
α-carotene	8/30	— —	<0.8–1.3	μg/dl
β-carotene	24/30	— —	<0.9–3.1	μg/dl
γ-tocopherol	ND[b]	— —	—	μg/dl
Lycopene	ND	— —	—	μg/dl
September (n = 21)				
Retinol	21/21	105.3 ± 7.6	47.1–176.2	μg/dl
Retinyl palmitate	7/21	— —	<0.7–37.4	μg/dl
α-tocopherol	21/21	2,289.3 ± 122.3	1,386–2,964	μg/dl
Lutein	21/21	10.68 ± 1.02	4.6–23.1	μg/dl
β-cryptoxanthin[a]	20/21	2.36 ± 1.02	<0.6–22.5	μg/dl
α-carotene	ND	— —	—	μg/dl
β-carotene[a]	19/21	3.15 ± 0.47	<0.9–9.2	μg/dl
γ-tocopherol	ND	— —	—	μg/dl
Lycopene	ND	— —	—	μg/dl

[a]Mean calculated using one-half the minimum detectable value for those samples with undetectable levels.
[b]Not detected.

DISCUSSION

The sex and physiological state of the bird must be considered when interpreting or comparing test results [Groscolas, 1982; Ghebremeskel and Williams, 1989; Ghebremeskel et al., 1991]. Fluctuations in levels of plasma retinol and α-tocopherol in relation to molting and breeding cycle have been documented for several penguin species [Ghebremeskel, 1988; Ghebremeskel et al., 1991; Monroe, 1993]. In this study, all the birds sampled were captured in burrows at the beginning of or during the breeding season. The sex of the birds sampled was not known, yet it is likely that the sample groups consisted of both male and female birds; some of the females were in lay or approaching egg laying, which could affect the mean and range of test values. In addition, numerous birds sampled in April were incubating eggs, while the birds sampled in September were just coming ashore and digging burrows, indicating that the April sample group was further into the breeding season. The physiologic changes that occur during breeding and egg laying, as lipids are mobilized and nutrients are carried through the blood to the developing egg, could explain the seasonal differences seen in both the assay means and in the number of samples that had detectable levels.

It must also be considered that the differences seen between the two study groups were due to locale. The two islands where the birds were captured are approximately 120 km apart, therefore the food and nutrient supply may be different.

When compared to other free-ranging species of penguin, the mean plasma retinol concentration found in this study is slightly lower than the mean values, but within the range reported, for prebreeding male macaroni penguins (*Eudyptes chrysolophus*), and slightly higher than that reported for prebreeding female macaroni

penguins [Ghebremeskel et al., 1991]. The mean was well within the range seen for both pre- and postmolt Rockhopper penguins (*Eudyptes crestatus*), but higher than that seen in pre-molt Magellanic (*Spheniscus demersus*) penguins [Ghebremeskel and Williams, 1989].

The mean plasma α-tocopherol concentrations were lower than those reported for pre- and postmolt free-ranging Rockhopper penguins [Ghebremeskel and Williams, 1988] and for prebreeding and premolt free-ranging macaroni penguins of either sex [Ghebremeskel et al., 1991]. Comparative plasma levels of retinyl palmitate, γ-tocopherol, and carotenoids in penguins were not found in the literature.

No significant difference was found between the mean retinol concentration of the free-ranging birds and that of healthy, captive Humboldt penguins housed at the Milwaukee County Zoo [117.9 ± 3.44 μg/dl, N = 209]. The mean α-tocopherol concentration of both sample groups was significantly lower ($P < 0.001$) than that found in the captive flock (3,587 ± 122.4 μg/dl, N = 209). The captive flock is fed a daily vitamin supplement containing 1,000 IU of vitamin A and 50 IU of vitamin E (Sea Tabs, Pacific Research Laboratories, Inc., El Cajon, CA). Lutein concentrations found in April were significantly lower ($P < 0.001$) than that of the Milwaukee County Zoo's captive flock (10.38 ± 0.53 μg/dl, N = 208), but the September mean was not significantly different.

Mean plasma concentrations of α-tocopherol and lutein showed significant differences between the two study groups. Therefore, in addition to comparing these results to those of the entire captive flock, only the values found in captive birds in March, April, September, and October were selected, and each month was averaged separately (April and September are the months the study was done, while March and October are the seasonal equivalents in the Northern hemisphere).

Mean α-tocopherol concentrations of both groups of wild penguins remained significantly lower ($P < 0.001$) than those found in the captive flock in March, April, and September; there were no October samples. Interestingly, the mean lutein concentration found in the April study group was not significantly different from those found in the captive flock in September (6.36 ± 1.28 μg/dl, N = 23), but was lower ($P < 0.001$) than those found in March (11.76 ± 0.9 μg/dl, N = 43) and April (8.93 ± 1.12 μg/dl). Conversely, the mean level found in the September study group was not significantly different from those found in the captive flock in March and April, yet was significantly higher ($P < 0.001$) than that found in the captive flock in September. This indicates that plasma lutein levels, and perhaps other carotenoids, may vary with the season and/or the physiologic state of the bird. Retinyl palmitate, β-cryptoxanthin, α-carotene, β-carotene, and γ-tocopherol were detected in 60.8%, 85.2%, 2.9%, 41.6%, and 16.7% of the captive samples, respectively. Lycopene has not been detected.

In some mammals, the presence of retinyl esters, such as retinyl palmitate, is an indication of vitamin A toxicity [Goodman, 1981]; however, the presence of retinyl esters has been documented in numerous species of mammals and birds without overt signs of vitamin A toxicity [Schweigert et al., 1990, 1991].

Carotenoids, such as lutein, β-cryptoxanthin, and α- and β-carotene, are speculated to have important roles in numerous physiologic functions, including the regulation of the growth and differentiation of epithelial tissues [Braun-Falco, 1981], protection of cells from photodamage, and involvement in reproduction [Krinsky, 1971]. The function varies with the carotenoid; some act as vitamin A precursors with

varying degrees of vitamin A activity, while others play a role in pigmentation, and give the egg yolk its yellow color. Birds are able to convert carotene to vitamin A but, unlike mammals, they preferentially store xanthophylls and other carotenoids in liver, egg, fat, skin, and feathers [Bauerfeind et al., 1971].

Although plasma values of α-tocopherol are a reliable indicator of both dietary vitamin E availability and the vitamin E status of an animal, the homeostatic mechanisms regulating plasma concentration of vitamin A render the interpretation of vitamin A status from plasma results less sensitive. Determination of hepatic vitamin A levels is necessary for more accurate conclusions about vitamin A status [Goodman, 1981].

Marked differences in serum α-tocopherol concentrations were seen in various species of raptors that were fed the same diet, which implies a species difference in vitamin absorption, metabolism, and excretion [Calle et al., 1989]. Therefore, to more accurately determine the nutritional status of penguins, baseline values for plasma vitamin concentrations should be determined for each species of penguin individually.

CONCLUSIONS

1. In free-ranging penguins, there were no significant differences between April and September in the mean plasma retinol concentration.
2. The mean plasma retinol concentration of free-ranging penguins was not significantly different from that of captive Humboldt penguins housed at the Milwaukee County Zoo, suggesting that the captive diets provide adequate amounts of vitamin A.
3. The mean α-tocopherol concentration is significantly lower than that found in captive Humboldt penguins housed at the Milwaukee County Zoo, suggesting that the captive diet provides more than adequate amounts of vitamin E.
4. Significant differences in the mean concentration of α-tocopherol and lutein were found between April and September; the means for both were higher in September.
5. The mean α-tocopherol concentration is lower than that reported for other species of free-ranging penguins.
6. Significant differences were found between the two sample groups in the number of samples with detectable amounts of retinyl palmitate, β-cryptoxanthin, and α-carotene. For retinyl palmitate and α-carotene the percentage was higher in April; for β-cryptoxanthin the percentage was higher in September; neither γ-tocopherol nor lycopene were detected in any sample.
7. Plasma lutein levels, and perhaps other carotenoids, may vary with the season and/or the physiologic state of the bird.

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