Testing a Vitamin Supplement Paste in Jackass Penguins (*Spheniscus demersus*)

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Introduction

According to the literature, piscivorous species fed dead, frozen fish in zoos have been shown to require supplemental vitamin E and thiamin (Engelhardt and Geraci, 1978, Robbins, 1993). The Rotterdam Zoo supplemented jackass penguins (*Spheniscus demersus*) with extra vitamins since 1991 by injecting liquid vitamins into the defrosted pilchards (*Sardina pilchardus*), mackerel (*Scomber scombrus*) and herring (*Clupea harangus*) fed to the birds. In 1996, Mazuri Fish Eater Tablets (Mazuri Zoo Foods, Essex, UK) replaced the liquid supplement, but were found to be more labor-intensive than the liquid supplement. A trial was initiated in 1999 to add a paste formula supplement (ThiaminE, Stuart Products, Inc., Bedford, TX, USA) to the defrosted herring and mackerel. Circulating vitamin concentrations were monitored before and after the supplement change. Implications of the resulting physiological parameters, as well as the method of supplementation, will be discussed and compared with the available literature.

Animals and Methods

The jackass penguin colony in the Rotterdam Zoo consists of 23 individuals (9.12.2) living in an outdoor exhibit. The land area comprises 50 m^2 while the basin comprises 45 m^2, with an average water depth of 0.6 m. The penguins breed regularly; at the moment the group contains six youngsters born in 1998, and three in 1999.

The natural diet of jackass penguins is 71% fish such as pilchards (*Sardinops ocellata*), maasbankers (*Brevoortia* spp.) and anchovies (*Engraulis japonicus*) (Gailey-Phipps, 1982). In Rotterdam Zoo, the penguins are fed by hand twice daily at 0730 and 1430 hr. Because no sardines have been available since 1996, the diet for the past three years consisted of 10% herring and 90% mackerel with an average consumption of 350 g of fish/day/bird. The fish are caught in the Irish Sea and the northern part of the Atlantic Ocean, frozen, and stored at -20°C. Fish are defrosted in running water for 8 hours during the day. After defrosting, half of the fish is fed in the afternoon and the other part is stored in a refrigerator until the following morning. Immediately before feedings, the fish are stirred with salt (0.5% w/w).

The fish are so called "low fat" fish, with an average length of 15 cm, and weigh between 75-150 g each. No analyses of the fed fish are made regularly; however, the Noorder Dierenpark measured 70 mg vitamin E and 0.6 mg thiamin (vitamin B_1) per kg in whole herring from the same lot as fed to the penguins in this trial (Berndt, personal communication). For mackerel, data were used from the Zootrition software program

(Wildlife Conservation Society, 1999). Vitamin supplementation (the former method of injecting liquid vitamins as well as later by adding the tablets) occurred 3 times a week (Monday, Wednesday and Friday). Every fish fed during the afternoon feeding time was supplemented with 1 cc of the liquid vitamin, or 1 tablet. On average, each penguin ate 2.45 kg fish a week supplemented with 9 ml of the liquid vitamin supplement or 9 Fish Eater Tablets. ThiaminE paste was added to all feedings at a dosage of 1 cc paste per kg fish (as-fed basis), applied and mixed through the fish manually. Each penguin received a calculated 2.45 ml (cc) of paste per week. Composition of the former liquid vitamins used, the tablets, and the ThiaminE paste, as well as the calculated intake of vitamins per kg fish can be found in Table 1. Data for analyzing the diets were obtained from the computer program Animal Nutritionist (N-Squared, Inc. 1987) and information provided by the manufacturers. The vitamin E in the Mazuri Fish Eater Tablets is listed as α-tocopherol (presumably d,l α-tocopheryl acetate, the most common synthetic feed form of this nutrient), whereas in the paste, the d-alpha-tocopheryl acetate (natural source) form is specified. The vitamin B_1 source in both products is thiamin mononitrate.

Table 1. Vitamin composition of subscribed liquid penguin vitamins (per ml), Mazuri Fish Eater Tablets (per tablet = 0.8 g), and the ThiaminE paste (per ml) per kg fish and the average calculated diet analyses after supplementation.

Nutrient	Liquid Pinguin vitamin	Diet analyses / kg	Mazuri vitamins	Diet analyses / kg	Paste	Diet analyses / kg
Vitamin E (IU)	31	160[1]	585	631[1]	100	146[1]
Folic acid (mg)			0.6	2.9	[2]	
Vitamin B1 (mg)	83	32.5[1]	183	185[1]	50	52.0[1]
Biotin (mg)			0.1		[2]	.
Pyridoxine (mg)			1.5		[2]	

[1] = included known data from fed fish: 90% mackerel and 10% herring
[2] = not specified by the manufacturer but included in the formulation

Circulating vitamin E and B_1 concentrations were measured in a random subset of the penguins (n=11) before ThiaminE paste supplementation, and repeated on the same birds 11 weeks post-paste supplementation. Blood samples were collected by venipuncture from the jungular vein using a 2 ml syringe and a 21 gauge needle, with dry heparin as the anticoagulant. While all bloodsamples were taken at 1100 hr, due to the difference in supplementation schedule, the pre-treatment samples represented concentrations 44 hrs after consuming supplemented fish (tablets) whereas the post-treatment samples represented blood concentrations 3 1/2 hrs after consuming supplemented fish (paste). Half an hour after bloodsampling the samples were stored minus 78 ^{0}C. After two days of storage they were send to the lab where they were defrosted and prepared for analyzing according the lab standard procedures.

Analyses were performed in the University Hospital of Rotterdam. If not enough blood was available, only vitamin E analyses were performed. Vitamin E (as α–tocopherol) was measured in heparin plasma after extraction with hexane using reversed phase HPLC and natural fluorescence detection (Driskel et al., 1982). Within and between run precision is generally better than 3.5% at 50 pmol. Vitamin B_1 was measured in whole blood using an HPLC-based method (Wielders and Mink, 1983). Within and between run precision amounted to less than 2% at about 100 nmol/l. The results of the assays can be found in Table 2.

Results

As can be seen in Table 1, supplementing with Mazuri Fish Eater Tablets at the specified dosage resulted in dietary vitamin E and thiamin concentrations four to six times higher than provided by supplementing with either the liquid or paste formulations.

The average plasma concentration of vitamin E at the beginning of the test period was 38.3 ± 12.3 µg/ml (mean ± s.d.), and after supplying the paste for 11 weeks, 37.7 ±7.5 µg/ml. Overall, the mean concentration remained stable and less variable following paste supplementation, with no significant difference noted in paired t-tests ($P = 0.45$, $n = 11$). All vitamin B_1 levels displayed an increase between the sample periods, averaging 63.9 ± 17.2 ng/ml pre-treatment to 153.9 ±30.9 ng/ml post-treatment with paste supplementation. Values which fell above the standard laboratory curve (concentrations > 101.2 ng/ml) were estimated linearly; unfortunately, samples were too small to dilute and repeat the assays. Even with only 5 samples measured pre- and post-treatment, differences were highly significant ($P < 0.001$) in paired t- comparisons.

Discussion

From the beginning of 1999, fish fed originated from the same catch. No analyses were done on vitamin B_1 and vitamin E levels in the fish itself, although estimated for (at least) the herring due to simultaneous assay by another facility. During processing and storage of fish and fish products, vitamin E levels have been shown to decrease concomitant with lipid peroxidation (Ruiter, 1995). Thiamin is heat labile, sensitive to oxygen, and easily degrades under conventional food processing conditions. Further, the concentration of thiamin in fish decreases through the presence of thiaminases which catalyze the decomposition of thiamin even during frozen storage (Ruiter, 1995). Hence even if vitamin E and vitamin B_1 concentrations are measured, single samples only represent momentary status, with little to no implications for vitamin concentrations over time. Nonetheless, Table 1 shows that there is a big difference in supplemented amounts of vitamins although the blood samples did not particularly reflect these differences. Possible reasons for a lack of direct correlation between the calculated dietary concentration, and circulating blood concentrations of these nutrients may include: differences in the stability of the vitamin supplements, absorption differences by the animals of the various vitamin preparations, inconsistent intake of the supplements (see application of vitamins below), or even the difference in time to supplementation compared with the measure in the circulation system.

Table 2: Analyses of the vitamin E and B_1 in blood samples of jackass penguins before ThiaminE paste supplementation, and 11 weeks following daily supplementation.

Sex F/M	Age Years[4]	Vitamine E µg/ml blood [1]	Vitamin B_1 ng/ml[1]	Vitamin E µg/ml [2]	Vitamin B_1 ng/ml [2]
F	29	36.4	62.4	38.9	180 [3]
M	13	43.4		38.2	167 [3]
F	10	24.8	79.6	27.5	195 [3]
F	6	35.3	37.8	33.1	99.5
M	5[4]	55.6	40.1	52.2	164 [3]
M	5[4]	64.4		51.4	
F	3	36.1	66.8	35.7	
M	0.5	44.5	83.3	40.3	149 [3]
F	0.5	30.0	77.6	33.5	
F	0.5	24.9	>101.2	32.1	122[3]
F	0.5	25.7		31.4	
Mean		38.3	63.9	37.7	153.9
s.d.		12.3	17.2	7.5	30.9

[1] before the paste was added to the fish
[2] after the paste 11 weeks was fed
[3] approximate amounts in ng/ml
[4]years in collection, birth date not known

It should be noted that this project has been held under practical circumstances. Although season and physiological state have been shown to influence feed intake as well as nutrient metabolism in penguins, which may be reflected in blood nutrient concentrations (Williams et al., 1989), no molting or breeding occurred in the penguins during this test period. Nonetheless it might be useful to repeat the trial in other seasons of the year.

Vitamin E

No difference was found in blood levels between supplementing the fish with tablets or paste, which may imply perhaps a difference in absorption and/or utilization of the various forms consumed. It is possible that the penguins better metabolized the natural-source vitamin E since circulating concentrations were equivalent despite almost a 6-fold difference in supplementation level. However, this study was not designed to specifically address that question, so interpretation must remain speculative. Nonetheless, considerably less vitamin E supplement in the paste form was required to maintain a similar circulating blood concentration of this nutrient compared with the tablet form.

Comparison of plasma vitamin E concentrations with data published from free-ranging penguins shows that values were within reported normal ranges (21.7 to 50.8 µg/ml; Williams et al., 1989). Circulating tocopherol levels summarized from free-ranging, as well as captive penguins (7 species) averaged about 24 µg/ml (Cosgrove and Dierenfeld, 1999). Compared to these previously summarized data, and published data on rockhopper penguins fed a known dietary level of vitamin E (mean 26.9 µg/ml; Maher and Dierenfeld, 1998), the values we found for jackass penguins are more than 50% higher. No comparative data for jackass penguins specifically have been previously reported, thus these values may be normal for the species.

Vitamin B_1

Captive piscivores such as penguins, herons, egrets, gulls, and storks, can develop a thiamin deficiency if fed dead fish is not supplemented with thiamin. Wild birds may not be exposed to a similar problem if they eat fresh fish, because thiaminases, the enzymes that break down thiamin, are compartmentalized and are only released upon cell death. Molds can also produce thiaminases, as well as several other thiamin antagonists which decrease the thiamin level in spoiled food, hence it is imperative to monitor fish quality closely. Lastly, the anticoccidial drug Amprolium has antagonistic activity against thiamin (Klasing, 1998), and must be taken into account in some captive situations.

Many factors influence the requirement for thiamin including size of the animal, composition of the diet, physical state of the animal, temperature of the environment, intestinal microflora, individual genetic factors, performance of muscular work, and age (Machlin, 1991). Most of the blood thiamin can be found in the leukocytes, although it can be found also in the red cells and in the serum. The level of thiamin in whole blood or its components has been extensively investigated as one possible measure of thiamin nutritive status. For this to be a reliable index, one must assume or prove that blood levels are a true reflection of tissue levels. This has been shown to be true in experimental animals but must still be assumed in humans (Machlin, 1991). Although hematocrit and transketolase activities were not measured in our test, it should perhaps be included in future studies to assist with data interpretation, and in order to get more reliable data on the vitamin B_1 status in animals.

No comparative values are available on the range of vitamin B_1 blood concentrations in jackass penguins to assess status. Adding vitamin B_1 in ThiaminE paste form to the fish increased blood concentrations considerably compared to supplementing with Fish Eater Tablets, although time to sampling (3 1/2 hr vs. 44 hr) could have been a major influencing variable due to the fact that thiamin is water soluble and not stored in the body. Additionally, other factors not quantified in this study are known to impact thiamin utilization in birds including physiological stage, genetic variation, weather conditions, fish quality parameters, stress, the presence of heavy metals, or deficiency or imbalances with minerals (i.e. Mg, Ca) or other vitamins (i.e. B_6, B_{12}, folate) (Machlin, 1991). Minimal blood levels for penguins in relation to vitamin B_1 status are not known, and more research is necessary to properly understand and evaluate vitamin B_1 metabolism in penguins.

Oversupplementation of this nutrient, however, does not appear to be problematic in captive feeding situations.

Applications of Vitamin Supplement

According the literature, supplementing fish with vitamins is necessary especially with regard to vitamin E and B_1. Supplementing can be done by injecting the fed fish, by putting tablets into the fish, or by applying a supplement (paste, powder) externally. Adding the recommended supplements is time consuming, however, especially with tablets and liquid vitamins. Supplementing the fish for 23 penguins requires 5-10 minutes by injecting, 10-15 minutes for tablets, and less than 1 minute for the paste application each time.

Advantages of a self-made liquid vitamin supplement are that extra vitamins can easily be added to the solution, and the cost of the basic components is low. However also some disadvantages should be noted. No information is available on the stability of the solution, especially on the stability of vitamin B_1. Regularly making the vitamin supplement is time consuming, as well as regularly injecting the fed fish with the vitamin solution. Further, and most importantly, liquid supplements are known to leak out of injected fish readily, and thus actual intake level is difficult or impossible to accurately quantify.

Advantages of adding tablet supplements include a long storage life, as well as a known quantity addition. Disadvantages often include the price, as well as the time entailed in adding tablets manually. According to the manufacturer's label, every penguin should receive one tablet a day, regardless of size of the bird or intake level. It can be logistically difficult to ensure single supplementation of individuals, and almost impossible to add extra vitamins in prescribed dosages. Additionally, sometimes the tablet supplements drop out of the fish, or are actively rejected by the consumer.

Advantages of adding a paste to the fish include a known dosage of stable quality, and less time manipulating the supplement. Supplying 1 ml of paste to every kg of fish can be readily accomplished by manual application of the correct dosage to the full amount of fish. Because it is less time consuming, every portion of fish can be supplemented with the paste daily. Further, the paste is water-insoluble, so does not dissolve even if fish are dropped or thrown into the water. Additional vitamin supplementation could be accomplished, if necessary, by mixing dry or liquid products into the basal paste. Some disadvantages: the paste is expensive. Additionally, consistent dosing by manual application may be unrealistic – it may stick to the sides of the bucket or even drop off the fish completely.

Conclusion

Although there are some disadvantages, adding a paste to defrosted fish is an easy and rapid method of supplementing fish with vitamins. ThiaminE maintained circulating vitamin E concentrations in penguins at a supplementation dose about 1/6 that of a tablet supplement, and resulted in increased circulating thiamin concentrations. While the current dosage formulation for ThiaminE may not be absolutely appropriate for all piscivores at this time, the concept, acceptance by the animals, and utilization by the keeper staff make this particular supplement an attractive product.

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