Association of Avian Veterinarians Australasian Committee Ltd.
Annual Conference 2015 23, 51-53

Demonstration of a dietary requirement of vitamin C in an Australian honeyeater, the Noisy Miner

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Abstract

Noisy miners (*Manorina melanocephala)* are nectarivorous honeyeaters found in Australia that eat a diet dependent upon Eucalyptus flowers and insects. Fourteen Noisy miners were placed on a diet deficient in ascorbic acid. Blood was collected weekly to monitor blood levels of vitamin C and other antioxidative agents, e.g., TEAC (trolox equivalent antioxidant capacity). It was difficult to entirely remove ascorbic acid from the diet, but after being on a diet deficient in ascorbic acid for about two months, the Noisy miners showed lesions of vitamin C deficiency including tibiotarsal fractures, vasculitis and feather abnormalities. At this point, the study was stopped and the birds returned to a balanced diet. This study has demonstrated that this species is reliant upon a dietary source of Vitamin C and is unable to endogenously manufacture this vitamin. Other antioxidants increased in response to the decline in blood levels of Vitamin C. This is the first Australian honeyeater that has been investigated to determine whether it can produce Vitamin C endogenously or requires a dietary source like humans, guinea pigs and rodents.

Introduction

A dietary requirement for vitamin C (L-ascorbic acid), a water-soluble vitamin, has been demonstrated in primates, guinea pigs and freshwater teleost fish. Most species can synthesise vitamin C from glucose using the glucuronic acid pathway. However, avian species that cannot synthesise vitamin C are usually insectivorous or frugivorous, where both dietary strategies presumably provide for sufficient dietary intake. Examples of Passeriform birds that lack the enzyme L-gulonolactone oxidase, and where a dietary requirement has been documented, include the Red-vented Bulbul (*Pycnonotus cafer*) and growing Willow Ptarmigan (*Lagopus lagopus*) chicks (Klasing, 1999). L-ascorbic acid may be formed in either or both the liver or kidney. Several avian species have been investigated to determine whether the L-gulonolactone oxidase enzyme is present in the liver or kidney. The change from kidney to liver production of this enzyme reflects evolutionary change. Reptiles produce ascorbic acid in the kidney, while mammals produce it in the liver. There is a mix between the two locations in avian species.

Ascorbic acid is required as an enzyme cofactor in collagen, carnitine and neurotransmitter synthesis pathways. It also acts as an antioxidant by scavenging reactive oxidative species (ROS) and is found in high levels in immune cells where it modulates phagocyte activity.

In the chicken, the increased requirements for an oxidative pathway in situations of stress such as heat, trauma, or infection may result in deficiency. The study involving Willow ptarmigan demonstrated clinical signs similar to scurvy in humans when dietary vitamin C was deficient. The birds showed poor growth, diarrhoea, lethargy and spontaneous fractures of the legs and wing bones (Hanssen et al., 1979). Red-vented bulbuls showed lethargy, feather loss and haemorrhages in the liver and leg joints (Roy and Guha (1958). Similarly in mammals, there may be lethargy, haemorrhages, poor skin healing.

In Australia, no species have been formally investigated to determine whether they have a dietary requirement for ascorbic acid, except for a preliminary study exploring carotenoid requirements over 28 days (Brian Rich, unpublished data, 2008) where low circulating levels of ascorbic acid were noted. In this study over 28 days, levels of ascorbic acid in the blood decreased when the concentration of ascorbic acid was reduced in the diet. The species investigated were Noisy miner, Blue-faced honeyeater (*Entomyzon cyanotis*), Purple-gaped honeyeater (*Lichenostomus cratitius*), White-fronted honeyeater (*Phylidonyris albifrons*), Crescent honeyeater (*Phylidonyris pyrrhopterus*) and White-naped honeyeater (*Melithreptus lunatus*). No clinical signs of avitaminosis C were noted in this study.

The Noisy miner has the dubious pleasure of being the only avian species to be listed federally as a threatening

process. At the same time, it is closely related to the Black-eared miner (*Manorina melanotis*) which is endangered. The Noisy miner is a bird of the open woodland of the eastern parts of Australia, including Tasmania, and has adapted well to the human changes to the landscape from urbanisation and agriculture. Its generalist diet is reported to be mainly invertebrates, nectar and fruit. Nectar is sourced from Eucalyptus, Grevillea, and Callistemon species as well as a variety of smaller shrubs, both introduced and native (Higgins et al., 2001).

Materials and methods.

Thirteen Noisy miners, which had been collected under permit at an earlier date, were identified with leg rings. They were housed in two aviaries 3m x 1.5m x 2m. Wombaroo® Lorikeet and Honeyeater food was fed as the sole diet for 14 days prior to dietary manipulation then aimed at reducing the concentration of ascorbic acid in the new diet. The original diet contained 280umol/L ascorbic acid, while the new diet contained 27 umol/L. Carotenoid levels in the diet were also reduced as part of another study. Water was available ad lib during the study. Each Noisy miner was caught, weighed and manually restrained for blood collection of 250uL from the jugular vein either weekly or fortnightly. Once collected, the blood was centrifuged at 37,000 relative centrifugal force for five minutes. The separated plasma was used for determination of d-α-tocopherol, retinol and total carotenoid by High Performance Liquid Chromatography. Ascorbic acid, TEAC, FRAP (ferric reducing ability of plasma), and urate were determined by chemical analysis using Roche Bio analyser.

Results

A significant decrease in mean plasma ascorbic acid concentration was observed after 36 days from 136 to 35um/L. There was also a significant decrease in the mean plasma d-α-tocopherol concentration which could suggest a sparing action by ascorbic acid for vitamin E and other antioxidants. There was a decreasing trend in the antioxidant index TEAC although the removal of circulating carotenoids may have contributed to this. The FRAP index remained relatively constant due to the consistency of urate concentrations which are a major contributor to this index.

As the experiment continued, the birds appeared to become physically weaker. This was readily seen by the ease by which they were captured for blood collection. The birds spent more time perching and were reluctant to fly.

Physical appearance of the Noisy miners remained unchanged until several weeks into the experiment. The most obvious change was fading of the yellow cheek skin of the bird, which reflected declining lutein levels in the blood. The beaks appeared to develop small petechiae and the skin over the featherless area of the neck was noted to have small petechial haemorrhages present at venipuncture.

At 32 days, two birds were found on the ground. Upon examination, both had fractures of the proximal tibiotarsal bone. In both cases the feather condition of the birds was considered to be poor, with new primary feathers showing pinching and abnormal growth. Both of the birds were thin, but it was not clear whether the weight loss was associated with ascorbic acid deficiency or an inability to reach the food bowls in the preceding 12 hours. The fractured tibiotarsus of each affected bird was stabilised using external coaptation. They were placed individually in a small cage for three weeks and returned to a diet that contained ascorbic acid, thus removing them from the study.

One bird was noted to be weak on one day at day 36 and was found dead the following morning. It was necropsied. No free blood was found in the abdominal cavity. Haemorrhages were found under the skull. Petechiation was seen on the skin and in association with feather quills. No fractures were seen. Recently moulted primary feathers showed broken shafts. The quills were easily plucked from the follicle. Tissues were submitted for histopathology and cause of death was attributed to intracranial haemorrhage.

Discussion

This is the first native Australian honeyeater species that has undergone a study where blood levels of ascorbic acid were determined after dietary manipulation of ascorbic acid. There is a possibility that other honeyeater species may also show a similar requirement for ascorbic acid. In light of this information, it is prudent to ensure that the diets offered to honeyeaters contains ascorbic acid at around 280umol/L.

Further work to confirm the inability to biosynthesise ascorbic acid in honeyeater species, such as the Noisy miner includes demonstrating the lack of either liver or kidney D-glucourono-reductase and L-gulono-oxidase, both of which are the enzymes involved in the conversion of D-glucuronolactone to L-ascorbic acid. Demonstration of the levels of ascorbic acid in the native food, such as nectar or insects which comprises the diet of Australian honeyeaters.

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