Title: Iron Storage Disease in Birds: Speculation on Etiology, Implications for Captive Husbandry

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Introduction

During the last three decades, aviculturists and nutritionists have become increasingly aware that iron, a critical nutrient for all vertebrates, can have toxic health effects on birds and that these effects vary from taxon to taxon. Storage of iron in the cells of the liver and other organs is part of normal metabolism and causes problems only if accumulation is excessive. In the 1970s, it was noted that, in particular, Birds of Paradise, Toucans and Mynahs died with livers marked by accumulation of iron. Over time, an increase in the number of facilities doing regular necropsies increased perception of iron storage as a serious problem. As awareness increased, iron storage disease was described from many other taxa. In the case of mynahs, problems could initially be traced to extremely high levels of iron in some common commercial diets. However, in other groups, iron-related problems occurred with diets containing what seemed to be moderate iron levels and mynahs continued to be vulnerable to iron storage, even on diets reformulated to lower iron levels. Identifying the causes of iron storage disease became a matter of serious concern.

What is iron storage disease?

Deposition of iron, in the form of hemosiderin, is normal in the liver, spleen, marrow and reticulocytes. Hemosiderosis is subjectively perceived or histopathologically quantified evidence of ‘more than expected’ deposition of hemosiderin, without accompanying clinical disease. Hemosiderosis has been reported from a wide range of species in zoo and private collections. In most taxa, iron load never reaches levels that cause health problems. In birds, the organs most often affected are the liver, heart and spleen. As iron levels increase, lysosomes are damaged and release ionic iron, which causes oxidative damage to membranes and proteins. Cells are damaged and replaced, tissues and organs can become fibrotic. We refer to this condition as iron storage disease. In human beings, the disease hemochromatosis, has been linked to a defect in the HLA locus of chromosome 6. The defect alters intestinal
uptake of iron at the basal level of the intestinal cell, causing liver damage from high levels of accumulated iron. In the past, hemosiderosis, iron storage disease and hemochromatosis have been used interchangeably, causing confusion. We will restrict the use of the term hemochromatosis to human medicine. Two hypotheses, genetic and dietary, have been suggested as explanations for the high incidence of hemosiderosis and iron storage disease in captive birds. Elements of both are persuasive.

**Genetic predisposition for iron storage disease**
The first explanation postulates a genetic basis for iron storage disease. In birds, genetic defects in individuals are not suspected but rather, gene-based differences among species in iron metabolism, especially in control of iron absorption.\textsuperscript{16,17} In birds, iron overloads are most commonly found in species that, in the wild, feed primarily on fruits and insects, especially fruit. Both insects and fruit are, in general, poor sources of dietary minerals. With natural diets low in iron, some bird species may have developed physiological mechanisms to extract dietary iron very efficiently. This hypothesis is supported by recent work. Mete et al,\textsuperscript{14} for example, demonstrated different mucosal transfer rates between doves and mynahs, taxa which also differ in susceptibility to iron storage disease. Mechanisms to compensate for scarcity of dietary iron might produce iron overloads when challenged with iron levels found in 'standard' captive diets.\textsuperscript{12,18}

**Dietary iron and iron storage**
The second hypothesis notes that iron levels in captive diets are generally much higher than available iron levels in wild diets. Most commercial diets have been formulated using mineral levels determined in studies of requirements for poultry. These levels range from about 50-120 mg/kg on a dry matter basis.\textsuperscript{19} However, it is difficult to create a diet with levels this low, from ingredients typically used for poultry feed. Commercial dry diets tend to have iron levels ranging from ~100-500 mg/kg.\textsuperscript{19,20} Galliformes have been reported with hemosiderosis, but not iron.
storage disease, so there has been no strong move to reduce iron levels in commercial feeds aimed primarily at the poultry market.

Iron levels reported on product packaging are usually calculated values, not guaranteed maxima and actual levels may be much higher. Apart from formulation, additional iron may be added inadvertently during manufacturing. In addition, such diet ingredients as mineral mixes and other supplements can contain significant iron contaminants that are not included in original calculations. The composition of any natural food varies, depending on soil, weather and other variables, including contaminants, so the composition of fresh ingredients, like fruits, vegetables and grains, may differ significantly from levels reported in food tables. A trend of increasing levels of iron in natural foods over time has been reported, as soils become contaminated with fertilizers and other chemicals, thus contributing a further source of unquantified iron. Hemosiderosis and iron storage disease in captive birds may simply be the result of chronic exposure to an excess of dietary iron.

Iron Metabolism

An understanding of basic iron metabolism is essential to any attempt to explain iron storage disease. Well over half of the iron present in a vertebrate body is in the form of hemoglobin or myoglobin, the molecules that transport oxygen. Total body iron is controlled by manipulation of the rate of absorption. Iron is absorbed inefficiently by all animals, although iron deficient animals can increase their rate of absorption of iron 3-10 fold. Once in the body, iron is extensively recycled, and losses are low. Reserve or storage iron occurs primarily in two chemically different, non-heme compounds, ferritin and hemosiderin. These are found throughout the body, but especially in the liver and spleen. In mammals, and probably also in birds, ferritin is normally the more common form, transported from the gut by the protein transferrin. However, the ratio of hemosiderin to ferritin increases as total iron stores increase. The rate at which iron is ingested also affects the form of stored iron. Hemosiderin increases as the availability or level of iron increases, perhaps because compounds used in manufacture of transferrin are limited.
in non-heme protein compounds must be released before it can be absorbed, while heme iron is absorbed directly, in the intestine. Very little free iron is found in the body, as free iron readily damages tissues.

Excellent reviews of iron in human and animal nutrition\textsuperscript{25,28} make it clear that the biochemistry of iron is extremely complex, involving many variables. Birds, as a group, have not been well studied, with most work done on rats, dogs and human beings. While some fundamental molecules and pathways are shared by birds and these mammal species, the differences seen among the mammals indicate that extreme caution must be taken when generalizing from a given mammal to birds, or even from one bird species to another. Doves and chickens, for example, have measurable differences in the structure of ferritin, an iron storage protein.\textsuperscript{29} In fact, within a single species, ferritin can vary in structure from one organ to another.\textsuperscript{30}

Efforts to develop animal models for study of the differential contribution of diet and physiology to hemosiderosis have determined that both must be considered. Dorrestein \textit{et al.}\textsuperscript{29} challenged doves (\textit{Streptopelia sp}), granivorous birds not ordinarily prone to hemosiderosis, with different doses of iron. They were able to show that doves could be used as a model for less available, more susceptible species, in studies of diet and hemosiderosis. Iron sensitive European starlings (\textit{Sturnus vulgaris}) fed purified diets trended towards increases in both liver iron and liver weight, with increases in dietary iron level, after as few as 60 days.\textsuperscript{7,31} Both studies showed an influence of dietary iron level. The rate and/or severity of deposition of hepatic iron, however, varies according to physiological mechanisms which are species specific. One shortfall of these studies is the assumption that acute exposure to very high iron levels accurately models longer term exposure to practical feed levels.

**Iron source and availability**

Total iron absorption is higher if meat is present in a meal than from non-heme foods alone, by a factor of three.\textsuperscript{22} If animal byproducts are used as a protein source for commercial diets fed to
frugivores and insectivores, not only may absolute iron levels in captive diets be higher than in natural diets, but also bioavailability. The interaction of higher dietary levels of iron and higher availability of dietary iron, plus higher rates of absorption by susceptible species, are probably enough to explain most occurrences of hemosiderosis and iron storage disease. However, other factors may also play a part.

**Interaction of iron with other minerals**

According to Morris:25 ‘The absorption of iron is affected by (1) the age, iron status and state of health of the animal … (2) conditions within the gastrointestinal tract; (3) the amount and chemical form of the iron ingested; and (4) the amount and proportions of various other components of the diet, both organic and inorganic.’ High dietary levels of manganese, copper, cobalt, cadmium and zinc decrease absorption of iron, evidently by competition for binding agents. High levels of dietary iron may interfere directly with absorption of copper, leading to secondary deficiencies of that nutrient. Thus, it is insufficient to look at the level of one mineral in a diet. The balance of minerals as a group must be considered (see also below interactions of dietary sugars and copper).

**Interactions with other components of diets**

Ascorbic acid (vitamin C) and other organic acids can increase absorption of iron from non-heme sources, but not from heme sources. However, if the organic acid is not consumed with the iron source, or if the non-heme iron is highly bio-available, this effect is not seen. Ascorbic acid enhances iron bioavailability, even in low iron diets. This is important, and deserves future study, as ascorbic acid occurs in many fruits and vegetables commonly fed to frugivorous birds. Vitamin C also functions as an antioxidant. Over the last two decades, there has been a trend for increased dietary supplementation with vitamin E, also an antioxidant. It is possible that increased vitamin E acts to spare dietary vitamin C, increasing its effect on iron availability with no change in dietary level. Improvement in product stability of vitamin C supplements in manufactured diets could produce a similar effect. Caution must be taken before lowering dietary levels of vitamin C,
however, as many birds have a requirement for vitamin C. Required levels are unknown, as is the magnitude of interaction with iron in different diets for different species. A ballpark recommendation for vitamin C in bird diets is 50-150 mg/kg (dry matter basis)\textsuperscript{22}.

The type of carbohydrate present can increase both iron absorption and iron retention in rats, through biochemical interactions with dietary copper. Lactose, sucrose, glucose and starch (as sources of dietary carbohydrates) display diminishing magnitudes of effect.\textsuperscript{32,33,34} In these studies, high fructose or sucrose are shown to generate free-radicals, which exacerbate deposition of iron in the liver. Starch and glucose do not have this effect. If iron physiology of birds is also affected differentially by different sugars (in association with dietary copper), that fact could have implications for which fruits should be used in diets for frugivores. It should also be noted here that wild fruits and domestic fruits differ substantially in nutrient composition, with many domestic fruits being far lower in minerals (see for example O'Brien \textit{et al.}\textsuperscript{35}).

**Polyphenols**

Polyphenols are common plant constituents that share the same core element, the phenol group. They contribute to color, taste, nutritional value and even structure of plants. There are many types and their chemistry is complex. Captive animals, in general, are fed diets low in phenols, regardless of the composition of their natural diets. In some cases, group names have been given to phenolic compounds with similar structure or chemistry, for example lignins and tannins. However, it is not unusual to find significant differences among members of one of these groups.\textsuperscript{36} Tannins, in particular, have been of interest as they are known to chelate, or bind strongly with, iron, possibly making it less bio-available. Tannins can be toxic and are among the group of compounds used by plants to defend against herbivores, so their use must be considered with caution. The tannins in tea, for example, can reduce the digestion of protein and inhibit appetite, while the caffeine in tea acts as a stimulant. Conversely, some tests have failed to show a reduction in the absorption of iron, with an increase in consumption of polyphenols.\textsuperscript{36}
Variation in susceptibility within and among species

There is apparently considerable individual variation within species, an indication that multiple factors contribute to hemosiderosis. Birds of Paradise, for example, are generally very susceptible to iron storage disease and elevated liver iron levels have been reported in chicks only a few weeks old. At least one Red Bird of Paradise at the Bronx Zoo, however, has lived on captive diets for >16 years, with no apparent detrimental effect. Hornbills are an order comprising species with diets ranging from carnivorous to frugivorous. While there has been general concern about hornbills and iron storage disease, preliminary information suggests that only the more frugivorous species are susceptible. In general, necropsy protocols for any species where iron storage is thought to be a consideration should include liver samples, either frozen or fixed in formalin, for mineral analyses and both nutritional and toxicological screens.

Diagnosis and treatment of iron storage disease

There are few, if any, clinical signs which diagnose iron storage disease and these generally occur at an end stage of the disease process. Common signs are labored breathing, abdominal swelling and ascites, weight loss and depression. On x-ray, enlargement of liver, heart or spleen is often seen. Blood tests may show high liver enzymes and hypoproteinemia. Unfortunately, none of these signs is restricted to iron storage disease. While high iron levels can be a cause of illness, they may also be a secondary effect of other diseases and can occur without clinical signs. Iron is often a limiting nutrient for pathogens and sequestering iron is an early step animals use in fighting infection by parasites or bacteria. An important part of the immune response is a large increase in the amount of transferrin produced by the liver, increasing the transport of circulating iron into tissues, especially the liver. In birds, stress also increases levels of transferrin and absorption of iron. In human beings, a battery of tests, including iron, ferretin and transferrin concentration, transferrin saturation and examination of ferritin receptors can differentiate among diseases of iron metabolism. While transferrin tests can be used in birds, diagnostic assays for ferritin have not yet been developed. At this time, a liver biopsy is needed for a confirmed antemortem diagnosis.
Treatments for hemochromatosis and iron storage disease have relied on repeated phlebotomy, to force utilization of stored iron.\textsuperscript{38,39} Another approach is to feed an iron deficient diet, taking care that deficiencies of other nutrients do not occur. A third approach uses a targeted iron chelating drug, such as deferoxamine\textsuperscript{39}. For birds that are not tame, repeated blood draws can be highly stressful. Iron deficient diets can work well, but it is essential to monitor food consumption, not food offered. Once a bird has suffered significant damage to internal organs, these remedies may come too late. For extremely vulnerable species like birds of paradise and Bali mynah, regular intervals on iron deficient diets could prove useful as prophylaxis.

**Recommendations**

Clearly, the problem of iron overload in avian species is extremely complicated and needs much further study. We cannot change metabolic differences but we can generate a series of recommendations for feeding iron sensitive species. It is important to get a lab analysis of any commercial diets used for iron sensitive species. It is neither possible, nor desirable, to create balanced, iron free diets for birds. All birds need some iron, to create blood and other tissues. Diets should be formulated to contain between 50 and 100 mg/kg iron, on a dry matter basis. Although 100 mg/kg may still prove high for sensitive species, this cannot be established without studies using controlled diets and it is difficult to create a balanced diet with iron levels substantially lower than \(\sim\)100 mg/kg. Some authors, however, have recommended levels of 50-65 mg/kg, for diets fed to susceptible species.\textsuperscript{18} Vertebrate products and byproducts should be eliminated or reduced in diets, as should foods fabricated with animal protein sources. Dietary vitamin C should be evaluated. The total diet should contain approximately 100 mg/kg of vitamin C\textsuperscript{22} but if possible, diet elements providing vitamin C should be fed at separate times from elements providing significant iron. Finally, further study of possible interactions of dietary sugars, copper, and iron metabolism should be undertaken in avian species.
Adding natural chelators, such as tannins, fiber and/or phytates, to diets for susceptible species has been a frequent suggestion. However, chelators can affect the entire complement of minerals in a diet, not necessarily in identical ways. In view of the complexity of interactions among minerals and other diet elements, chelators and other active compounds should not be indiscriminately added to diets until appropriate studies have been undertaken. A targeted chelator like desferoxamine may successfully remove excess iron but has not been extensively investigated in avian species. A high priority for captive bird collections should be support of controlled studies to examine dietary iron and methods to protect iron sensitive species.

Summary

Both genetics and diet contribute to the development of hemosiderosis and iron storage disease in birds. Sensitive species have more efficient mechanisms of iron absorption. Factors such as iron level, iron source and interactions with other compounds all effect the availability of dietary iron. Diagnosis is difficult and treatment is limited.

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