DETERMINATION OF TANNIN LEVELS IN VARIOUS PLANTS AND THEIR POSSIBLE EFFECT ON IRON CHELATION IN LEMURS

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Extended Abstract

Lemurs and Iron Storage Disease

Lemurs are one of several species that can suffer from excess iron accumulation in tissues, termed hemosiderosis. The more severe form, hemochromatosis, develops when hemosiderosis is accompanied by functional or morphologic evidence of iron toxicity and may result in clinical disease and death.14 Organs most often affected include the liver, heart, spleen, and gastrointestinal tract. The pathology of this problem/disease is not entirely understood although it has been discussed by many scientists and veterinarians. Hemosiderin is deposited in multiple organs including the duodenum, stomach, jejunum, ileum, liver, spleen, kidney and lymph nodes. Excessive iron deposition can have toxic effects on parenchymal cells as well as hepatocytes. Histologic lesions reported in the liver include fibrosis, hepatocellular necrosis, distorted architecture, bile duct hyperplasia, hepatocellular adenoma, hepatocellular carcinoma, and cholangioma is also reported.1

Hemochromatosis, also known as Iron Storage Disease (ISD), can be caused by excessive dietary iron intake, excessive iron uptake from the gastrointestinal tract, hemolysis, or inadequate excretion of iron.14 As serum iron levels increase, lysosomes release ionic iron, damaging membranes. Cells are damaged and replaced; tissues and organs can become fibrotic, leading to ISD.18

Hemosiderosis has been reported in a wide range of species (birds, primates, fruit bats, hyrax, rhinoceros, horses, and pigs) in zoos and private collections.21,22 Iron overload has also been described in several other species of primates including New World monkeys, gorillas, and lemurs.3 Captive lemur species reported to have hemosiderosis and pathologic findings associated with iron overload include the black and white ruffed lemur (Varecia variegata variegata), ring-tailed lemur (Lemur catta), black lemur (Eulemur macaco), brown lemur (Eulemur fulvus), crowned lemur (Eulemur coronatus), and red ruffed lemur (Varecia rubra).22

In captivity, lemurs progressively accumulate iron deposits in a variety of organs throughout their lives. In one study black and white ruffed lemurs were analyzed to survey serum iron, total iron binding capacity, transferrin saturation, and serum ferritin levels. Data analysis showed no differences in these analytes attributable to sex, but significantly higher levels of serum iron, transferrin saturation, and serum ferritin in older animals.6 As the animal ages, hemosiderosis is
more prevalent and developing hemochromatosis is a higher risk. Three publications in the 1980's reported an incidence of hemosiderosis ranging from 69% to 100% in lemurs over 1 year of age.\textsuperscript{3,9,10,22} The risk of iron overload in captive lemurs is still discussed however; little new information has been provided about this condition since the 1980's.\textsuperscript{9} Either genetic or dietary factors, or a combination of both, may lead to the reported high incidence of hemosiderosis and ISD in captive animals.\textsuperscript{18} Hemosiderosis is the result of a complex process involving diet; the absorption, metabolism, and excretion of iron; and disease processes.\textsuperscript{5}

**Dietary Conditions and Iron Storage Disease**

Captive diets may be higher in iron, as well as in ascorbic acid, which enhances iron absorption from the intestine. Natural diets may contain high levels of tannins, which chelate iron in the gastrointestinal tract and decrease intestinal absorption.\textsuperscript{1} Excessive iron absorption could also be a result of inadequate binding or chelation of dietary iron by tannins and other natural chemicals.\textsuperscript{5} Alternatively, natural browse could contain iron chelators that bind dietary iron, making it less available to free-ranging species. Similarly, tannins in rainforest water might bind dietary iron so it is less bioavailable. A lack of iron chelating compounds in food and water in captivity might predispose animals to excessive iron absorption, producing eventual hemosiderosis and hemochromatosis.\textsuperscript{5}

It has been suggested that tannins, a group of phenolic compounds, can be used as a primary dietary additive to reduce risk of ISD in captive lemurs. Tannins are naturally-occurring secondary metabolites of plants belonging to the phenolic class that contribute to the color, structure, chemical properties, nutritive value and taste of plant products.\textsuperscript{15} They are found in a wide range of plant species; high levels of tannins have been found in grape skin and seeds, sorghum, walnut hulls, tea, and some berries.\textsuperscript{2}

Tannins can form complexes with protein and carbohydrate; they can also form complexes with minerals such as iron. Tannins can function as antioxidants either by direct OH\textsuperscript{-} scavenging or by iron chelation.\textsuperscript{13} Tannins can reduce the bioavailability of iron by binding to it as a “natural chelator,” and causing iron to be excreted.\textsuperscript{17}

Historically, tannins have not been incorporated into the diets of captive animals because they have been referred to as anti-nutritional. According to researchers, condensed tannins are considered to have a negative effect leading to nutritional complications because tannins bind to essential minerals and lower digestive enzyme activity.\textsuperscript{19}

Although behavioral responses are somewhat equivocal, tannins do not seem to be strongly selected against when found in native foods eaten by many species, and a number of animals have developed physiological mechanisms for dealing with high levels of dietary tannins.\textsuperscript{16} Some wild animal species, such as lemurs, that ingest large amounts of dietary tannins have developed a defense against tannin overload via the secretion of praline-rich salivary proteins that selectively bind the tannin.\textsuperscript{15} In captive lemurs, hemosiderosis is linked to a diet containing little or no tannin as well as high ascorbic acid levels.\textsuperscript{12} A number of animal species, including wild lemurs, that ingest large amounts of tannin have developed a defense against tannin overload via secretion of proline-rich salivary proteins that selectively bind the tannins.\textsuperscript{15}
Tannins may play a huge role in the metabolism of iron and the overall health of wild animals with ISD. Therefore, incorporating tannins into the diets of captive animals, prone to this disorder, is logical. The goals of this project are to determine if tannins truly have an effect on lemur iron absorption and storage, to determine tannin palatability with lemurs, and to find an inexpensive and abundant source of tannins.

**Development and Testing of a Tannin Tablet**

To determine the effect of tannins, a tablet has been developed to add to the diurnal lemur diet. Using pure tannic acid allows for accuracy and precision for testing the effects on iron storage and excretion. The tablet ingredients include 8% pure tannic acid, cellulose, corn syrup (keeps the tablet together), fructose, gelatin corn oil, magnesium stearate (for tablet quality), silicone dioxide (for tablet quality), and orange oil. The orange oil is aromatic and helps with palatability. The nutrients of the tablet, as fed, are 6.75% protein, 2% fat, 59.9% fiber, 1.5 ppm iron.

The tablet will be added to the diet of five ring-tailed lemurs (*Lemur catta*) and five black lemurs (*Eulemur macaco*) at the Saint Louis Zoo. Each animal will act as its own control. Pre-project, as well as every 30 days, each animal will receive a complete physical examination including body weight, and have blood collected for complete blood count and serum biochemical profile to assess health status. Each animal will also have an iron analyte panel performed, as validated for lemurs.

This panel includes assessment of serum iron, ferritin, transferrin saturation, and total iron binding capacity. These parameters have been determined for these species of lemurs in captivity and in the wild. Serum trace minerals will also be monitored. This will be one of the most important features of this project. Adding tannins may chelate some minerals, and the risk of trace mineral deficiencies must be prevented.

Fecal matter will be analyzed using several antioxidant, tannin, and iron methods. In order to measure antioxidant activity ABTS, Folin, and high pressure liquid chromatography (HPLC) methods will be used and for measurement of tannin content a Vanillin-HCl and HPLC method will be used.

Noninvasive methods of evaluating systemic iron levels include assessing the serum indices of iron metabolism: serum iron, total iron-binding capacity (TIBC), transferrin saturation, and serum ferritin. Serum ferritin is generally accepted to be the best indirect predictor of iron stores in most mammalian species studied. Serum ferritin concentration will be measured with a recently developed enzyme-linked immunosorbent assay (ELISA) using polyclonal anti-human ferritin antibodies adapted to measure black and white ruffed lemur ferritin.

**Tannin Sources**

Determining sources, other than a purified tannic acid, is valuable for monetary and convenience purposes. Sources being tested include red sorghum bran (*Sorghum bicolor*); it’s used as a control during tests because it has been shown to contain an average of 8 mg of tannin per gram. Various red grape pomaces (the skins and seeds left over from wine pressings) including Norton grapes (*Vitis aestivalis*), Concord grapes (*Vitis labrusca*), Chambourcin grapes (*Vitis vinifera*),
Vincent grapes and Saint Vincent grapes. The last tannin source tested will be ground black walnut hulls (*Juglans nigra*). Each of these plants, highly abundant and obtainable, are considered high sources of antioxidant activity and tannin content.

The tannin tablet being used in the lemur diet is primarily for the use of testing tannin iron binding capacity. The tablet is made with purified tannic acid which is expensive. Grape pomace and walnut hulls may provide a useful alternative and can easily be incorporated in the diet. The pomace and walnut hulls can be ground and added to a tablet, in place of the tannic acid, once tannin inclusion rate is determined, and palatability will be addressed as done with the purified tannin tablet (possibly using orange oil).

Although previous dietary changes in the formulation of primate diets have reduced the degree of hemosiderosis found in lemurs, and nearly eliminated hemochromatosis, further changes must be implemented to determine the effect of tannins on iron. This research will determine how tannins affect dietary iron absorption and iron stores in lemurs, the palatability of a tannin tablet, and alternative, inexpensive sources of tannins available for use. These determinations will help to improve the overall health and life of captive lemurs. The information may also prove useful in further investigations for tannin incorporation into diet of other captive species at risk for hemosiderosis and hemochromatosis.

**LITERATURE CITED**


