THE EFFECT OF LENGTH OF COLD STORAGE ON SECONDARY PLANT COMPOUNDS IN BROWSE FOR CAPTIVE BLACK RHINOCEROS (*DICEROS BICORNIS*)

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Abstract

The endangered black rhinoceros (*Diceros bicornis*) suffers from a high rate of mortality in captivity due to conditions such as hemosiderosis and hemolytic anemia. Supplementation of the diet with native browse species rich in tannins and other iron-binding polyphenolics has been proposed as a dietary strategy for reducing iron absorption and accumulation in this population. This study examined the effect of cold storage versus fresh material on iron binding polyphenolic (IBP) and iron binding tannin (IBT) contents of a North American browse species, prairie sumac (*Rhus lanceolata*). Leaves and stems of prairie sumac were collected fresh or stored in a cooler (-15°C) for up to one year. Fresh and frozen samples were analyzed for total IBP and IBT content. IBP and IBT levels in both stored and fresh leaves reached peak accumulation at day 21. IBP and IBT in stored leaves demonstrated significant decreases thereafter until day 70 (2.32 mgE gallic acid/g dry matter and 1.42 mgE gallic acid/g dry matter, respectively); no further significant changes in IBP content observed from days 70 to 365. No statistically significant differences in IBP or IBT over time were detected in or between stored and fresh stems. Though there were significant changes in the leaves, after day 70, the concentrations tended to be similar to that of the stems (1 – 2 mg equivalents gallic acid/gram dry plant). These findings indicate that cold storage of this browse species is a possible option for dietary supplementation of iron-binding compounds to captive black rhinoceros to reduce iron accumulation in this population.

Introduction

The term polyphenolic is used to describe a wide variety of plant secondary compounds that are not essential for the life function of the plant. Tannins are a subclass of polyphenolic compounds that have the ability to precipitate alkaloids and some proteins.[6] Tannins can be categorized into two major types: condensed tannins (proanthocyanidins modified by esterification or by oxidation) and hydrolysable tannins which are easily broken down into smaller polyphenolic compounds.[5,6]

Browses contain levels of polyphenolic compounds that, while possibly limiting the digestibility and availability of some nutrients, may have other beneficial attributes.[6,10] Polyphenolics may function as iron-chelators and thus as anti-oxidants.[3,8,17] An iron/polyphenolic complex will not react with oxygen, whereas reduced iron will bind to

molecular oxygen to form highly reactive free radicals. These free radicals may cause cellular lipid peroxidation.[6,17] Polyphenolics that have not been absorbed from the digestive tract may also protect proteins or other biomolecules in the tract from oxidative damage.[6] In one study, quebracho (*Schinopsis balansae*), which contains polyphenolics in the form of condensed tannins, led to a significant increase in fecal antioxidant status when supplemented to black rhinos.[2] Polyphenolics may also bind to iron in the digestive tract of animals, preventing the absorption and bioavailability of iron and potentially decreasing the occurrence of diseases such as hemosiderosis or hemochromatosis.[6,7,9,11,14]

The endangered black rhinoceros (*Diceros bicorni*) is extremely rare in its native habitat and intensive management of the black rhino in captivity attempts to ensure its survival. Many institutions feed the black rhinoceros a diet of grasses and/or hays supplemented with a nutrient-dense complete feed. It has been suggested that iron overload in captive black rhinoceroses[7,9,11,14] may be related to the absence of high polyphenolic browse species regularly consumed in its natural habitat.

Consequently, it may be important to feed or supplement captive black rhinos with polyphenolic-rich browses throughout the year. In many areas, seasonal availability precludes the collection of fresh browse year-round. In order to provide consistent browse supplementation, institutions may need to collect and store browse in available coolers or freezers for up to a year. Previous work has indicated that leaves may be stored at -20ºC for three months without major changes in the content of individual tannins, however, storage at room temperature (25 ºC) leads to decreased levels of individual tannins.[12] Conversely, a pronounced loss of polyphenolics was observed with storage at -23 ºC (67% loss at three months, 88% loss at six months), however, losses in polyphenolic content were much less at a storage temperature of -70 ºC (11% loss at three months, 12% loss at six months).[1]

Therefore, the objective of this study was to determine whether there would be a change in the iron binding polyphenolic (IBP) and iron binding tannin (IBT) content in a common North American browse species, prairie sumac (*Rhus lanceolata),* when stored in typical cooler conditions (-15ºC) over a period of time.

Materials and Methods

Plant material collection

Collection of prairie sumac (*Rhus lanceolata)* occurred in the summer, 2004, at Fossil Rim Wildlife Center in Glen Rose, Texas. Sumac was collected from three locations throughout the park. Locations chosen were those typically harvested for browse fed to the institution's black rhinoceros. Collection did not minimize leaf loss, but rather occurred as it would under normal conditions. At day zero, approximately 40 representative branches were harvested from each of three locations throughout the park. Five of these branches were brought, in a cooler, to the Fort Worth Zoo Nutritional Services laboratory (FWZL) for immediate processing and analysis. The remaining branches were stored in sealed trash bags in a cooler at -15°C. At days seven, 14, and

21, five branches were collected randomly from the three locations (same location at each collection) throughout Fossil Rim and five branches were sampled from those previously harvested and then stored in trash bags in the cooler. Branches were immediately placed in coolers and brought to the FWZL for processing and analysis. After day 21, fresh browse was no longer harvested. Five branches were removed from the freezer at each of days 70, 98, 126, and 365, and brought to FWZL for processing and analysis.

Plant material processing

All processing and analysis took place as soon as the browse samples were brought to the FWZL, approximately two hours after harvesting or sampling from the cooler. Leaves were immediately separated from stems and both portions were ground into a homogenous powder with liquid nitrogen. Samples were ground in a commercially available blender (Robot Coupe Blixer®, BX6V™, Ridgeland, MS, 39157 or Hamilton Beach®, 990™, Washington, NC, 27889).

Polyphenolic/Tannin analysis

Because of the hypothesis that polyphenolics and tannins bind to iron and therefore eliminate excessive loads of iron in the diets of black rhinoceros, it was decided to follow a method of analysis specific for IBP and IBT.[4]

Leaf and stem samples were analyzed in duplicate for IBP and IBT according to a protein precipitation method previously described.[4] IBP and IBT samples were read at λ_{max} 510 nm on a Beckman DU520® general purpose UV/Vis spectrometer (Beckman Coulter™, Inc. Fullerton, CA 92834). Concentrations were calculated using a standard curve with gallic acid as the reference compound.

Statistical analysis

A two-factor ANOVA with repeated measures on both factors was performed on fresh vs. stored browse, dry matter, location, time, and any interactions that may have occurred over time. A one-way ANOVA for independent samples was conducted to determine an effect of location on stored vs. fresh or the data over time. A one-way ANOVA was also conducted to detect a significant change that may have occurred within the study period. Data are presented as mean $\pm$ standard error (SEM). Level of significance was set at five percent. (VasserStats: Website for Statistical Computation ©Richard Lowry, 1998-2007, Poughkeepsie, NY).

Results

In order to eliminate any effects of dehydration over time, all data are reported on a dry matter basis. A significant loss of water ($P< 0.05$) occurred in stored leaves (Table 2), however, no significant losses occurred in stems. There was a significant change in the level of polyphenolics and tannins in stored and fresh leaves over time. Specifically, a significant increase occurred in the level of polyphenolics and tannins in stored and fresh leaves from day zero to day 21. Fresh leaves were collected until only day 21 and

therefore polyphenolic levels were compared to stored leaves for the first 21 days only (Figures 1 and 2). There were no significant differences between stored and fresh leaves over the first 21 days, however, there tended to be an effect of time noted on the increase in polyphenolic compounds in stored versus fresh leaves during this period ($p = 0.058$). After reaching peak levels at day 21, polyphenolics and tannins in stored leaves decreased significantly from day 21 to day 70. No further significant changes in polyphenolic content occurred from days 70 to 365. In contrast to leaves, there were no statistical differences found in the levels of polyphenolics or tannins over time in fresh versus stored stems (Tables 1 and 2).

Discussion

In the current study, there was a significant increase in the level of IBP and IBT in leaves collected fresh over a 21 day period. Seasonal[13] or plant variation may have caused this change. Though fresh leaves were sampled from the same area on each collection day, the samples were collected randomly and may have not been from the same tree. It is interesting to note that stored samples also had a significant increase in IBP and IBT over the first 21 day period. These leaves were collected on the same day (day zero) and subsequently sampled for analysis. Dry matter corrections were conducted to eliminate any effect of desiccation and therefore it is possible that leaves follow similar trends of increase whether fresh or stored.

The change in season precluded the ability to continue to collect fresh leaves; however, stored leaves were subsampled and analyzed over a period of a year. After day 21, there was a significant decrease in the level of both IBP and IBT in stored leaves, specifically between days 21 and 70. This period of time to significant polyphenolic loss is shorter when compared to previous studies.[1,12] The rapid loss of polyphenolics in this study may have been due to differences in storage temperature, species and type of plant material used, or study design and methodology. Comparisons of numeric results using different methodologies should be made with caution, however, the decrease in IBP and IBT seen in this study at 21 days goes along walnuts stored at room temp (25C) for 21 days (20-40% reduction).[15] It may be that temperatures well below freezing are necessary to maintain levels above two mg equivalents gallic acid/gram dry plant.[1]

There were no statistically different increases or decreases in the levels of IBP or IBT in fresh or stored stems over the period of time in this study. It may be worthwhile to look into the feeding of stems as a more stable option for polyphenolic supplementation. Though there were significant changes in the leaves, after day 70, the concentrations tended to be similar to that of the stems (one – two mg equivalents gallic acid/gram dry plant). Further research is necessary to determine levels of polyphenolics that may reduce the occurrence of excessive iron stores in the captive black rhinoceros population, however, it may be a viable option for institutions to store browse at -15°C in order to feed polyphenolic and tannin-rich browse year-round.

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Table 1: Dry matter (%DM) and levels of iron binding polyphenolics (IBP) and iron binding tannins (IBT) on a dry matter basis basis ± standard error (n=3) in fresh leaves and stems of *Rhus lanceolata* over a period of 21 days.

		Fresh leaves		Fresh stems		
		mgE gallic acid/g dry plant				
Collection date (day)	%DM	IBP	IBT	%DM	IBP	IBT
07-27-04 (D0)	40.66	0.66±0.45[a]	0.44±0.33[a]	55.59	1.13±0.61	0.60±0.34
08-03-04 (D7)	42.90	1.43±0.11[ab]	0.88±0.12[a]	44.93	0.37±0.04	0.17±0.03
08-10-04 (D14)	46.58	4.16±1.47[b]	2.84±1.13[ab]	51.51	1.33±0.23	0.51±0.11
08-17-04 (D21)	50.18	4.57±0.89[b]	3.67±0.74[b]	55.05	1.27±0.17	0.69±0.15

[ab]Numbers within columns with different letters are significantly different (P<0.05). No letters are reported for stems because no significant differences were found.

Table 2: Dry matter (%DM) and levels of iron binding polyphenolics (IBP) and iron binding tannins (IBT) on a dry matter basis ± standard error (n=3) in stored leaves and stems of *Rhus lanceolata* over a period of 365 days.

		Stored leaves		Stored stems		
		mgE gallic acid/g dry plant				
Date sampled from cooler	%DM	IBP	IBT	%DM	IBP	IBT
07-27-04 (D0)	40.66	0.66±0.45[ad]	0.44±0.33[ad]	55.59	1.13±0.61	0.60±0.34
08-03-04 (D7)	49.32	1.01±0.06[af]	0.82±0.02[ae]	47.90	0.26±0.07	0.11±0.02
08-10-04 (D14)	53.99	3.35±0.70[abe]	2.36±0.65[abf]	46.94	1.30±0.08	0.53±0.05
08-17-04 (D21)	62.27	5.91±1.27[b]	4.30±0.86[b]	51.25	1.27±0.19	0.56±0.08
10-05-04 (D70)	82.19	2.32±0.07[ce]	1.42±0.19[cf]	44.17	1.49±0.25	0.57±0.34
11-20-04 (D98)	84.71	1.65±0.15[cdf]	1.09±0.11[cdef]	50.11	1.07±0.24	0.56±0.17
11-30-04 (D126)	84.40	1.81±0.22[cf]	1.32±0.18[cef]	65.97	1.11±0.38	0.76±0.37
07-25-05 (D365)	86.34	2.27±0.38[ce]	1.44±0.20[cf]	77.18	1.27±0.31	0.72±0.14

[abcdef]Numbers within columns with different letters are significantly different (P<0.05). No letters are reported for stems because no significant differences were found.

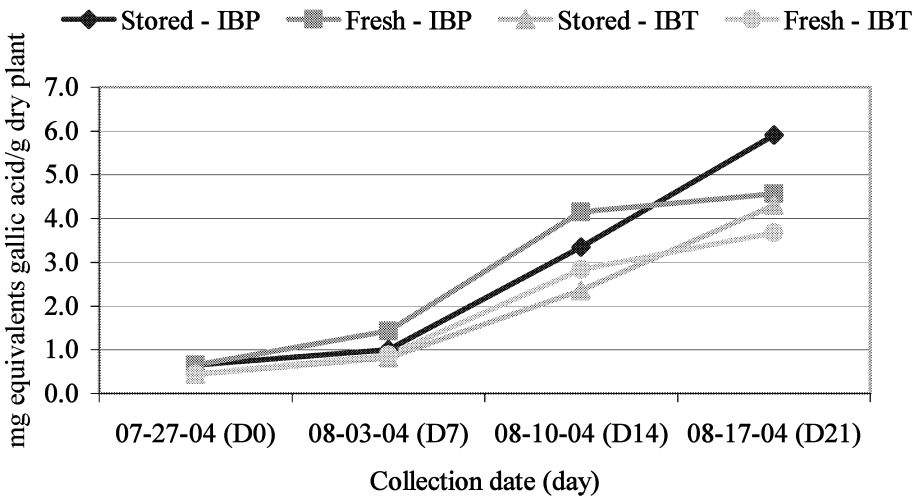

Figure 1: Levels of iron binding polyphenolics (IBP) and iron binding tannins (IBT) in *Rhus lanceolata* on a dry matter basis in fresh and stored (-15°C) browse leaves (n=3) over a 21 day period

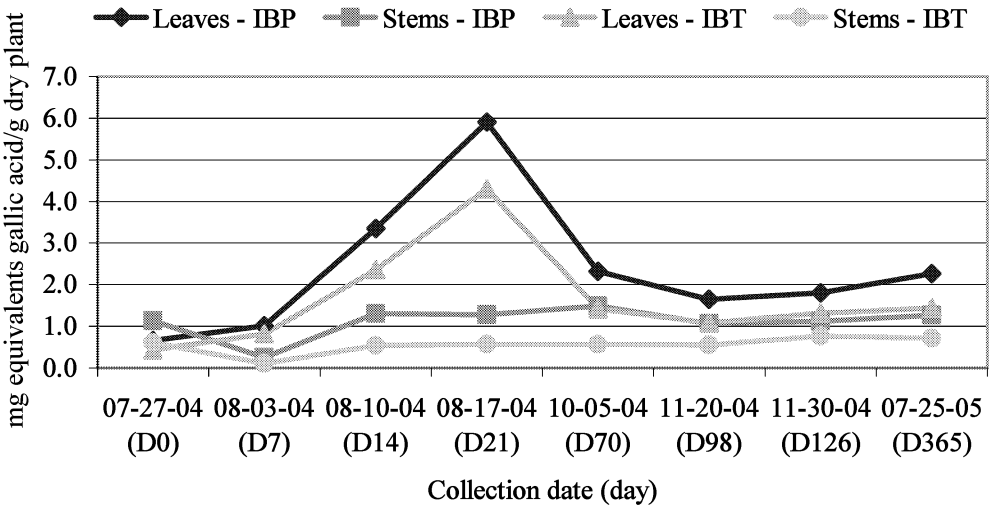

Figure 2: Levels of iron binding polyphenolics (IBP) and iron binding tannins (IBT) in *Rhus lanceolata* on a dry matter basis in fresh and stored (-15°C) browse leaves and stems(n=3) over a 365 day period

CARP CAKES: ANOTHER FISH ALTERNATIVE

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Extended Abstract

Introduction

Silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*H. nobilis*) are invasive species that pose a threat of displacing and lowering the abundance of native fishes, mussels and invertebrates.[10] The Mississippi Interstate Cooperative Resource Association (MICRA) considers bighead and silver carp the most important aquatic nuisance species in the basin.[4] On the Missouri, Mississippi and Illinois rivers, bighead and silver carp populations have the potential to double in a year.[14] Environmental threats from high populations of silver carp include competition for food with native planktivores and larval fish of nearly all species, and also changes in water quality.[11] Bighead carp can reach sizes up to 50 kg and silver carp can reach up to 27.3 kg.[14]

Since carp are prolific and grow to large sizes they have the potential to provide an economical alternative as food for piscivorous species in zoos, thus reducing harvest of marine fish and assisting in clearing non-native fishes from local waterways.

The development of silver-carp-based diets for zoo animals was initiated. Since the size of silver carp precluded simply feeding the whole fish to the animals, development of a product made from ground, whole, uncooked carp was begun. Early steps in the project involved collaborating with the University of Missouri-Columbia, U.S. Geological Survey, Missouri Department of Natural Resources, Missouri Department of Conservation, and the Native Fish Conservancy to create a recipe mimicking the size and nutritional composition of whole fish. Whole silver carp were ground and formed into a processed "cake" using a cold gel set process developed by food scientist Dr. Andrew Clarke of the University of Missouri-Colombia.[5] Fat-soluble vitamins A and E were extracted and proximate nutrient composition (water, protein, fat, ash) analyzed, along with mineral and fatty acid profiles of cold set gel carp product, before the addition of Vitamins B and E. In addition to nutrient content, carp cake texture is extremely important for acceptability by piscivores; hence measurements were taken for cohesion, as well as "tossability" (for feeding program application). Prototypes were soaked in water for up to two hours to ensure they would remain cohesive. Supplemental levels of B vitamins and vitamin E will be added to a final prototype. The final product will be tested in a pilot feeding trial with penguins, pelicans, and sea lions at the St. Louis Zoo and other participating facilities in 2007. Palatability and intake, physiological status of the animals (including weights and body condition), and