Rhinoceros Nutrition Overview Ellen S. Dierenfeld, PhD, CNS

Keeper Workshop - Disney's Animal Kingdom, May 1999

### **Background History**

At a meeting held at White Oak Conservation Center in the early 1990s, the chemical composition of native forages consumed by black rhinos was summarized as the basis for evaluating captive diets fed to these species. On the basis of approximately 7 field studies for which chemical data were reported, captive diets, in general, were found to be high in crude as well as available protein, and likely to be deficient in fatty acids, linolenic acid in particular. Although no data were reported on the soluble carbohydrate content of native browses. fibrous carbohydrate components offered in captive feeding programs were felt to be, in general, less lignified and highly digestible. Mineral imbalances were suggested both in native forages (low Zn, Se, Na, P) as well as in captivity (particularly high Ca). Further, vitamin E was found to be much higher in native forages than dry zoo diets, and carotenoids were suggested as a possible important source of vitamin A. All major nutrient categories were found to be in need of improvement or better understanding in feeding captive rhinos. Recommendations from this summary included: 1) feed mixed grass and legume hay rather than either as the sole forage source (to balance out some of the nutrients), 2) supply proper carbohydrates (less digestible) and fats (essential fatty acids), 3) investigate the mineral status of rhinos and native browses in more detail and 4) re-evaluate fat-soluble vitamin supplementation. It appeared, at the time, that vitamin E was being supplemented adequately but that circulating vitamin A levels in captive animals were higher than in free-ranging animals.

A number of diseases with possible nutritional links were highlighted with respect to these various nutrient entities including: 1) hemolytic anemia – vitamin E deficiency was originally felt to underlie this syndrome, but was since shown to be not associated; however, overall poor antioxidant status may have a link with other diseases. 2) Ulcerative dermatitis – was suggested as possibly due to glucose imbalance, amino acid deficiency, specific fatty acid deficiency, and/or mineral imbalances. 3) Hemosiderosis – reported in black rhinos was felt to be due to mineral imbalances, particularly Fe and Cu. 4) Overall impaired immune function – could be due to any or all combinations of nutrient imbalances suggested above. 5) More recently (1998) a new disease entity was identified -- peripheral vasculitis – with a possible regional proclivity, suggested as linked to vitamin C deficiency, antioxidant imbalance in general, and/or singly or in combination with mineral and fatty acid imbalances.

How can the keeper contribute to achieving the goal of maintaining healthy captive rhino populations? One important means is to stay abreast of current research questions, controversies, and questions in the feeding of zoo rhino populations. Another is to be aware of, and follow, current feeding recommendations for the species. If you don't understand the rationale underlying specific recommendations, ask – take the initiative to query your Supervisor, your Curator, the Nutrition Advisor. Do some background reading yourself, and familiarize yourself with the current literature, become informed and take responsibility for meeting the needs of the species under your care. Your attendance at this meeting shows a great commitment towards meeting that goal, and we appreciate your participation and interest.

More Recent Investigations

A number of ongoing nutritional studies have been conducted over the past 5 years to fill in some of the gaps in our knowledge of rhinoceros nutrition, and will be summarized briefly.

### Fat Soluble Nutrients

A survey of fat-soluble vitamin constituents, funded by the International Rhino Foundation, looked at retinol, carotenoids, vitamin D, and E in tissues and blood samples from 4 species of rhinos. Elephants and domestic horse samples were compared as possible physiologic models. Samples from the US tissue bank in St. Louis, and 28 blood samples from free-ranging Zimbabwean rhinos supplied by Dr. David Jessup (also funded by IRF) were used in this evaluation. Retinol (a measure of vitamin A activity) was found to average  $0.03 \pm 0.01 \mu g/ml$  in free-ranging black rhinos, compared with  $0.10 \pm 0.10$  in captive black rhinos. No carotenoids were

detected in any blood samples, free-ranging or captive. The mean found in the free-ranging animals is about 10 times lower than most domestic animals, and probably represents a physiologic normal for this species. We are possibly feeding diets containing excessive vitamin A to captive black rhinos.

Free-ranging black rhinos have vitamin E (tocopherol) concentrations ranging from 0.2 to 0.8  $\mu$ g/ml, depending upon habitat, and captive animals currently are being supplemented and have achieved a mean concentration of 0.7  $\mu$ g/ml, which is not different from that seen in captive white (0.06  $\mu$ g/ml) or Asian (0.7  $\mu$ g/ml) rhinos. Overall, rhinoceros do not have high circulating concentrations of this nutrient, and attempting to duplicate levels in domestic herbivores (1 to 3  $\mu$ g/ml) does not appear warranted. We still do not understand vitamin E metabolism in rhinoceros. They have no high density lipoprotein fraction in their plasma, which is responsible for vitamin E transport in other species, and the absence or presence of tocopherol transport protein in the liver is currently under investigation (detection and sequencing) by Dr. Maret Traber at Oregon State University (funded by IRF).

# Protein

As far as protein investigations, tyrosine deposits have been identified as cellular inclusions in rhinoceros by Harley et al at the University of Capetown (1997). The significance of this finding, is at this time, unclear. Salivary binding proteins, a possible ecological adaptation for dealing with diets high in condensed tannins, have been detected in captive black rhinos in Australia (Neiper, 1998), and is being followed up with other captive and free-ranging rhinoceros samples by Fickel in Berlin. Dave Jessup has submitted free-ranging black and white rhino plasma samples for amino acid determination, to provide baseline normal data for comparison with samples collected on zoo rhinos.

Condensed tannins have been measured in Zimbabwean and North American browses by Wright (1998) and found (by relative score) to be about 10 times higher in browses than typical diets fed to rhinos in North American zoos. Further work on tannins in Texas browses is being conducted by Ward (Ft. Worth Zoo) and Pond (Texas A&M). Woodfine and others (University of Kent & Zimbabwe) are looking at tannins in native browses, as well as investigating total and bound protein fractions.

# Fiber, Energy & Digestive Physiology

Several intake and digestion trials with browsing rhinos have been conducted over the past 5 years, or are in process. Atkinson (University of Harare, 1996) fed rhinos in bomas in Zimbabwe with native browses, and found diets to be 30 to 50% digestible. Sumatran rhino (n=3) trials were conducted at Cincinnati zoo (1997) and diets were found to be up to 80% digestible. A further digestion trial with native browses was conducted in Malaysia with Sumatran rhinos in 1998 (Kilbourne et al), and results of that study are pending. With funding support from IRF, intake and digestion trials for captive black rhinos are being conducted in the UK, Germany, Australia & Japan in 1999, as well as in a Zimbabwean semi-reserve. Diet quality is also being assessed for a number of rhinos on reserves in South Africa, with funding from the St. Louis Zoo and IRF (Adcock). Although intake and digestion trials are time consuming and require extra work (weighing and collecting all feed, refusals, and feces), the information gleaned from these studies is essential. Often times, success or failure of these types of studies hinges directly upon support and assistance from the keeper staff.

# Fatty Acids

Several fatty acid investigations have been conducted, including an evaluation of native and North American browse composition by Wright (1998). Briefly, she found that fresh browses contained 15 times higher concentrations of linolenic acid compared with linoleic acid, but that oxidation of linolenic acid initiated as soon as browse was cut (undetectable within 20 minutes). Further, she looked at fatty acids in zoo diets compared with browses, and found linoleic acid concentrations 5-fold higher, and linolenic acid concentrations to be one-third those found in fresh browses. These findings may have health implications for browsing rhinos. In a survey of 20 adipose tissue samples evaluated from dead zoo rhinos, using fatty acid concentrations as a measure of long-term diet effects. Dierenfeld and Frank (1998) found that 25% of the animals were deficient in linoleic acid, and more than 50% had undetectable levels of linolenic acid.

Linoleic acid (omega-6 cicosanoids) have been linked with proinflammatory responses, allergies, arthritis, psoriasis, and colitis in other species, while linolenic acid (omega-3 cicosanoids) have the opposite effects. Imbalances in the omega 6:omega 3 ratio in the diet is associated with immune system disorders, neurologic disorders, problems with carbohydrate metabolism, joint abnormalities, cardiovascular irregularities, and cancers in humans and study species. Clinical trials with dietary supplementation of linolenic acid (flax-based product) in zoo rhinos has shown a positive response in altering plasma omega 6:omega 3 ratios (Suedmeyer and Dierenfeld, 1998). Longer term studies, with more animals in several institutions, is underway. Additionally, fatty acid ratios are being documented from free-ranging rhino plasma samples (IRF funded), and fatty acid composition of adipose tissue in a biopsy specimen of a living rhino has recently been obtained.

### Minerals

Mineral investigations include plasma and tissue concentrations from 4 species of rhinos, with elephants and horses as the physiological models (funded by IRF). Samples from the US tissue bank in St. Louis, and 28 blood samples from free-ranging Zimbabwean rhinos supplied by Dr. David Jessup (also funded by IRF) were used in this evaluation. Blood work is currently underway, but the liver samples have been processed. Preliminary data showed that many animals (particularly black rhino) had excess Ca, Fe, and Se in the liver, and Zn, Cu and Mo interactions were apparent. Additionally, heavy metals (As, Pb, and Cd) were detected in some samples, which were detailed to staff at the respective facilities for followup. The horse was used as the physiologic model for the minerals evaluated. Na, K, Mo, Zn, and P all appear to be within horse normal ranges for all rhino species. No normal range for sulfur was found for horses, but all rhino species displayed liver S concentrations between 2000 and 3000 mg/kg. Se and Ca were higher than horse normal ranges for black rhinos only, and Cu appeared excessive compared to horses in the grazing rhinos (whites and Asian), but not the browsers. Fe concentration was high for all for rhino species compared with domestic horses: half of the black rhinos displayed liver Fe concentrations 20 times higher than the overall rhino mean.

Ferretin saturation is being evaluated in a number of black rhinos, particularly those displaying tissue iron deposition (Paglia et al, this conference and elsewhere). High Fe diets are suggested to lead to hemosiderosis, and/or trigger an oxidative damage cascade metabolically. Suggestions that natural browse-based diets high in tannins, phytates, and fiber constituents that may bind Fe to make it less available, and associated health benefits to captive browsing rhinos, are being pursued through an integration of many of the studies outlined here. Mineral investigations, particularly with respect to the idiopathic hemorraghic syndrome reported in black rhinos, are being conducted by Frederick (University of Arizona) and Ward (Fort Worth Zoo) and Pond (Texas A&M). Marginal trace mineral status (especially of Zn and Cu) is known in the southwest, and may contribute to this problem. Detailed diet evaluations are underway. Copper is particularly targeted as a key component of several enzyme systems including ceruloplasmin (involved in Fe metabolism), lysyl oxidase, superoxide dismutase, cytochrome C oxidase, tyrosinae, ascorbate oxidase, catalase, desaturase (fatty acid metabolism), and neuropeptide release.

Dietary and fecal mineral concentrations in free-ranging rhinos are being investigated by Adcock in South Africa, and Woodfine and others in Zimbabwe, funded by the St. Louis Zoo and IRF. Understanding the basis and interactions of these minerals will likely be crucial in alleviating some of the problems that may be induced by diet. Captive Diets Alterations

Finally, optimal captive diet development is being investigated through development of several commercial products attempting to combine the information gathered through these investigations to supply proper balances of major nutrients, minerals, vitamins, and fatty acids. Many of these products are incorporating native or substitute browses to supply protein, carbohydrate, and other chemical constituents that possibly can't be duplicated by refined ingredients. We hope these integrated efforts will rapidly move us forward in improving the captive feeding management of browsing rhinos. Several years ago, a project was discussed that involved ranking browses fed to rhinoceros in zoos through a series of palatability and nutrient composition trials. This idea should be revived, and championed, by the keeper staff caring for the browsing rhinos. Input, suggestions, and feedback directly from the animal care staff involved are critical in success of all these studies. As you're well aware, switching animals to new diets often involves extra time and effort, but the rewards can be more than short-lived. In the case of the browsing rhinos, we feel that proper diet is key to very survival of the species.