

## **Rhino Nutrition Update**

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Although a general overview of current topics in rhinoceros feeding and nutrition will be addressed, specific questions raised by AAZK Keepers are also targeted in this review. The AZA Rhinoceros Taxon Nutrition Advisory Group currently comprises 4 members: Michael Schlegel (Disney's Animal Kingdom), Kerrin Grant (Utah State University), Marcus Clauss (University of Zurich) and Ellen Dierenfeld (Saint Louis Zoo), focusing on white, Asiatic one-horned, black and Sumatran rhino species, respectively. Over the past decade, much attention has focused on nutritional concerns within the Rhinocerotidae due to potential links with a number of diseases – particularly within black rhinos – including:

- hemosiderosis (possibly due to mineral imbalances)
- hemolytic anemia (associated with vitamin E and/or antioxidant status)
- ulcerative dermatitis (may be linked with glucose, amino acid, fatty acid, and/or mineral imbalances)
- peripheral vasculitis (suspected vitamin C, general antioxidant, fatty acid and/or mineral issue)
- Overall impaired immune function is also suspected in the Rhinocerotidae

Current rhino research updates highlight publications covering mineral nutrition, description of a novel antioxidant metabolism, considerations of tannin-binding salivary proteins, intake trials in Indian rhinos, and ongoing field studies. While feeding habits certainly differ, at this time, there is no solid data to suggest differences in nutrient requirements among rhino species. Further, the domestic horse appears to be the most appropriate physiologic nutrition model.

### **Minerals in Rhinos**

A broad survey of mineral concentrations in tissues and blood of rhinoceros (Dierenfeld et al., 2005) examined serum/plasma concentrations in 4 species of rhinoceros in US zoos (n=34 blacks, n=3 to 16 whites, n=3 Indian, and n=3 Sumatrans) as well as free-ranging rhinos (n=27 blacks, n=5 whites). Additionally, liver tissues from 21 blacks, 6 whites, and 2 to 4 Indian and Sumatran rhinos were also analyzed. Based on these data, the horse appears to be an excellent physiologic model of mineral metabolism in rhinoceros. Captive rhinos, however, displayed higher circulating Ca and Se, and lower Na concentrations compared with domestic equids. The browsing rhinos showed high Fe concentrations in both blood and liver tissues, as well as low hepatic Cu content. Conversely, grazing rhino species had higher Cu concentrations compared with equids. Captive black rhinos had higher circulating levels of Fe than free-ranging animals; additionally, females

showed higher concentrations compared with males. Liver K, Mg, Co and Mo concentrations all showed a significant increase with age in rhinos.

Excess iron storage detected in browsing rhinos has been suggested as linked with low dietary tannin levels, since tannins are known to bind iron and make it less bioavailable. Additionally, tannins possess antioxidant activity. It appears, however, from combined mineral data that diet alone is not the only underlying factor in the high incidence of hemosiderosis reported in (particularly) the browsing rhino species.

Lack of dietary fiber may also be associated with an increased bioavailability of dietary iron – fiber, both soluble and insoluble – is known to bind Fe in other species, but effects have not been investigated in detail in rhinos. A further nutrient that may impact iron bioavailability is dietary vitamin C, which is known to increase Fe uptake both *in vivo* and *in vitro* (Gaffney et al., 2004). Citrus fruit in captive rhinos diets likely has little to no effect on Fe absorption – native browses and many green plants eaten by rhinos likely contain higher ascorbic acid levels than would the occasional fruit consumed. A key aspect to remember in mineral nutrition is the high degree of interaction – rarely do any nutrients (and particularly minerals) work in isolation, thus one must consider multiple factors.

### **Unique Antioxidant Metabolism**

Another unique system, with possible antioxidant function, has been identified in rhinoceros through biochemical laboratories in South Africa (Weber et al., 2004). The amino acid tyrosine has been found in rhinoceros red blood cells (but not plasma) at concentrations 50-fold higher than found in humans. Researchers have also determined that tyrosine content is elevated in other Perissodactyla species, including equids and tapirs. Within the rhino species, captive animals had significantly lower levels compared with free-ranging animals, and much variation was seen across species. The working hypothesis is that elevated tyrosine functions as a novel antioxidant system in rhinoceros, but further work is needed to confirm this pathway/mechanism.

### **Salivary Tannins and Rhinos**

Within the animal kingdom, salivary tannin-binding proteins (TBP) are related to dietary habits, with browsers showing the highest concentration.

TBP

were examined in 3 rhinoceros species, reported in a recent publication (Clauss et al., 2005a). Saliva samples from grazers (white rhinos, n=9), browsers (black, n=10), or mixed (Indian, n=8) feeders were compared, using both hydrolysable (tannic acid) and condensed (quebracho) tannin standards. Black rhino saliva bound both hydrolysable and condensed tannins to a considerably higher degree than white rhino saliva. Indian rhino saliva, however, showed binding capacity with hydrolysable tannins that was equivalent to black rhinos, but very high capacity for binding condensed

tannins (higher than in blacks). Differences between black and white rhino saliva were expected due to the variation in feeding habits between these species. Results from Indian rhinos, however, suggest that this species may have more recently switched from browsing to grazing habits – still retaining capacity for adapting to dietary secondary compounds that may or may not be present in current diets.

Salivary TBP are also inducible in rhino species; since browsing rhinos (and especially blacks) eat tannins in nature, seasonal dietary habits might benefit from inducible mechanisms for dealing with these compounds. Six rhinos at 3 zoological facilities were fed for 3 mo diets containing either no tannins (typical zoo diet) or diet + 5% tannic acid or diet + 5% quebracho. A significant increase in tannic-acid binding capacity was detected with both tannic acid and quebracho feeding. In contrast, a non-significant trend was reported for quebracho-binding capacity with quebracho feeding, no change was found with tannic acid feeding. These results suggest that hydrolysable tannins may play a greater role in the native environment of black rhinos compared with condensed tannins, but native foods need to be looked at in more chemical detail. This information may also link with iron storage disease and natural mechanisms for binding dietary iron; feeding trials are ongoing in both European and US zoos to examine Fe balance with and without added dietary tannins.

### **Indian Rhino Nutrition**

Chronic foot problems and leiomyomas, possibly linked with excess body weight, led to intake, passage, and digestion trials conducted on 11 Indian rhinos in 4 zoos in the US and Europe (Class et al., 2005b). Diets with or without concentrates were fed; in addition to total intake and fecal output, Ca balance and water intake were measured. Zoo diets comprised a variety of grass or mixed hays or silage (one zoo fed straw!), along with herbivore pellets, produce, and some mineral supplements. Rhinos consumed 0.5 to 1.1% of body weight in dry matter (DM) daily, eating only 0.27 to 0.99 MJ digestible energy / kg body weight<sup>0.75</sup> (compared with an estimated requirement of 0.5 – 0.7 from standard equations). Seven of 11 rhinos consumed more energy than needed, even when fed a roughage-only diet. DM digestibility ranged from >60% to about 20%, decreasing with increasing fiber. Digestibility was expected to decrease when concentrate was removed from diet, but actually did not change in one of three facilities. It may be necessary to restrict both concentrate and forage portions of the diet in this species to maintain optimal body condition. *Ad libitum* hay is possible as a diet for Indian rhino, but a mineral/vitamin supplement may be needed to balance out possible nutrient deficiencies.

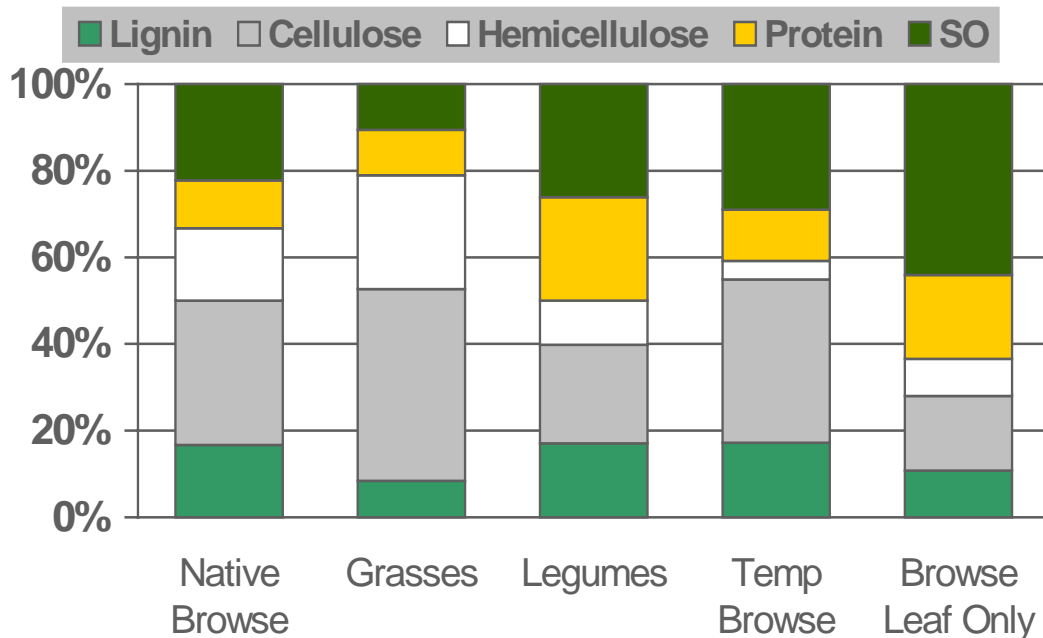
Water intake was found to be equivalent to domestic horses, approximately 30-49 ml/kg body weight daily. Calcium metabolism was found to be similar to horses and rabbits – not dependent on vitamin D, and excreted through the urine. As a consequence, bladder and kidney stones

(Ca-based) are commonly reported in rabbits and horses, but have not been found in rhinos. Further mineral investigation is warranted in rhinos, but it appears from this and other studies that the horse is a good model of mineral nutrition.

### Optimal Forages for Rhinos (5 slides – starts 30)

The most critical element of any herbivore diet remains the forage source. Analysis of native browses eaten by browsing rhinos, compared with typical grass and legume hays fed in US zoos, are shown in Figure 1. Also displayed are selected temperate browse species and leaves only of the same browses (data from Dierenfeld et al., 1995, Nijboer and Dierenfeld, 1996, and Dierenfeld, unpublished). Browses – either native or temperate – contain an intermediate fiber content compared to grass (higher) or legume (lower) hays, with fiber content defined as hemicellulose, cellulose, and lignin combined. Legumes and browse leaves alone contain higher protein levels than forages typically consumed by browsing rhinos, since rhinos eat the woody fractions of plants as well as leaves in nature. These types of chemical comparisons are necessary in order to identify the most appropriate forage substitutes for captive feeding programs, particularly when native browses are not available.

Figure 1. Composition of forages eaten by rhinoceros species.



Although grasses appear to contain a more appropriate protein and fiber content than legumes (such as alfalfa), the soluble carbohydrate fraction (listed in Figure 1 as SO, or Soluble Organics) in particular, may be important. These soluble carbohydrates may be a good source of soluble fiber for lower

gut fermentation for energy and requires further investigation. Studies have shown that many browses have a more rapid rate of fermentation and digestion than many grasses – this may also be important for optimal gut function in rhinos. By nutrient composition, a good quality grass forage and a proper concentrate pellet, in a ratio of 6:1 (grass:pellet by weight) can meet maintenance nutrient requirements for rhinos, as currently defined.

Browse can most certainly have behavioral enrichment value in feeding programs, even if the nutrient profiles may not be fully optimal. Browse should be kept as palatable and fresh as possible – it is unclear at this time whether or not maintaining the roots on browse plants will maintain nutrient profiles. Certain nutrients have been shown to degrade rapidly with cutting of the plant (such as vitamin E, fatty acids), whereas other nutrient levels (minerals, fiber, protein) remain rather stable. Taken together, current information suggests that alfalfa should NOT be considered an optimal substitute forage for browsing rhinos, and increased intake of grasses and/or mixed grass/legume forages should be encouraged.

Alfalfa is quite high in protein (nitrogen), calcium, and magnesium content compared to recommended dietary nutrient profiles for rhinos. Additionally, the calcium to phosphorus ratio is quite skewed (very high in calcium compared to phosphorus), and hypophosphotemia has been reported as a health issue in browsing rhinos (see Table 1). In other species, alfalfa is associated with increased hindgut pH – which may alter passage rate and digestibility. Alfalfa is also quite digestible in itself – much more so than native rhino browses. Finally, alfalfa may be associated with salivary buffering issues in browsing herbivores; acid/base imbalances are more prevalent in hoofstock consuming alfalfa compared with grass forages.

Table 1. Select minerals (DM basis) in typical grass compared with alfalfa hays (all early cut).

Nutrient	Timothy	Orchardgrass	Alfalfa
Ca, %	0.5	0.3	1.6
P, %	0.3	0.3	0.2
Mg, %	0.1	0.1	0.4

Grass has also been occasionally reported problematic for rhinos, with at least one case of grass staggers reported with white rhinos grazing perennial ryegrass (Bluett et al., 2004). This health issue is due to a fungal endophyte that contains alkaloids, localized in the leaf base or seed heads – thus both overgrazing or undergrazing pastures may contribute to incidence of the problem. Clinically, rhinos display tremors, lethargy, stagger, and may collapse. Specific tests can be made to determine the absence or presence of the alkaloid (lolitrem B, toxic at concentrations >1 mg/kg DM), or simply

avoiding pastures or hay containing perennial ryegrass may be best defense against this potential hazard.

### **Pregnancy Diets**

As with all mammals, no increased dietary nutrient requirements are necessary to support reproduction until the last trimester and during lactation periods, during which times calorie (energy) needs about double, protein requirements increase from about 8-10% (DM basis) to 12-14%), Ca increases from 0.3 to 0.5%, and P, 0.2 to 0.3%. If the basal diet already contains these levels of nutrients, clearly increases may not be needed. It is best to alter the overall digestibility of the diet to meet these increased requirements during reproduction, rather than simply adding more quantity (due to physical limitations of the digestive tract in processing high amounts). In this case, adding browse and/or legume (alfalfa) forage will add protein and Ca – possibly targeted concentrates may be added if needed. It is highly recommended that the basal diet be evaluated prior to any change; overnutrition is as detrimental – or more so – than undernutrition during pregnancy, and may be associated with dystocias as seen in other animal models. There is also limited evidence that overnutrition, particularly of certain nutrients, may contribute to skewed sex ratio in other hoofstock species.

### **Fat-Soluble Vitamins**

A recent publication measured circulating levels of vitamin E in free-ranging black rhinos in South Africa (Ndondo et al. 2004a). The authors reported 9 animals with values ranging from 0.19 to 1.92 µg/ml alpha-tocopherol; 3 animals had undetectable levels. The overall mean of 0.86 µg/ml was similar to levels reported in other studies with free-ranging rhinos where means ranged from 0.24 (Kenya, n=7) to 0.80 (Namibia, n=3) (Clauss et al., 2002). Vitamins A and E are currently higher in captive black rhinos compared to free-ranging animals that have been examined (see Table 2; from Clauss et al., 2002), and horses do not appear a good physiologic model for fat-soluble vitamins in rhinos. The table data also suggest a difference in values pre- and post- 1990 – perhaps due to differences in supplementation before and after that time period.

Table 2. Plasma fat-soluble vitamins in black rhinos.

µg/ml	Vitamin A (Retinol)	Vitamin E (alpha-tocopherol)
Captives (n=85)	0.08 ± 0.08	0.84 ± 0.96

Pre/Post 1990	0.09 / 0.08	0.46 / 1.03
Free-ranging (n=136)	0.04 ± 0.03	0.58 ± 0.30
Horse	0.18-0.35	2.0 – 10.0

Vitamin E concentrations in browses eaten by black rhinos ranged from 100 to 729 µg/g DM in leaves only (Ndondo et al., 2004b) from South Africa, and were 59 to 341 µg/g DM in browses from Zimbabwe (n=26; Dierenfeld et al., 1995). Based on available information of dietary measurements and animal response, it is recommended that all rhino species consume diets containing at minimum 50 IU vitamin E/kg DM, up to about 200 IU/kg DM. While not a daily dosage per se, if rhinos eat approximately 1% of body mass, a 2000 kg black rhino will eat about 20 kg DM (X 50 IU per kg DM) or about 1000 IU vitamin E per day. Status should be evaluated at least annually via blood concentration measurement.

An isolated vitamin D toxicity incident was reported with 3 black rhinos in 2 facilities in 2002. Two captive animals had blood levels of 25(OH)D3 >160 ng/ml; one animal displayed a value of 28 ng/ml. All had mineralized soft tissues suggestive of vitamin D toxicity. Normal captive animals have blood levels of about 28 ± 4 ng/ml, whereas free-ranging rhinos (n=28) had values of 56 ± 34 ng/ml. In this case, feed manufacturers were very responsive to the situation, and tested all hold-back feed lots; however, a specific diet problem was not isolated in this case and no further cases have been reported (Miller, pers. comm., 2003).

### **Fatty Acid Nutrition**

A seasonal shift in fatty acids has been reported in black rhino browses sampled from South Africa, with increased polyunsaturated fatty acids (PUFAs) – particularly C18 – in winter vs. spring plants (Ndondo et al., 2004b). These authors found no relationship between fatty acids and vitamin E status, but noted that fats were essential for proper vitamin E absorption. Other studies have documented evidence of fatty acid deficiency in black rhinos. From studies of browse composition in Zimbabwe and North America, Grant et al. (2002) detected that fresh browse contained alpha-linolenic acid (an n-3 essential fatty acid) at concentrations about 15 – fold higher than linoleic acid (another essential fatty acid, n-6). Immediate oxidation was detected upon cutting the browse, with about 90% degradation of linolenic acid within about 20 minutes. Hence, if the animal is not eating fresh from the shrub, the chemical content and nutritional value of browse can be radically altered. By comparing zoo diets with native browses, Grant et al. found that zoo diets contained 5 times more linoleic, and 1/3 the linolenic acid, consumed by these browsing rhinos in nature. These alterations may have health consequences.

Investigations with zoo rhinos showed a positive clinical response in terms of n-3 to n-6 ratios in black rhinos by adding a flax-based dietary fatty acid supplement; typical zoo diets had the opposite effect on these ratios (Suedmeyer and Dierenfeld, 1998). Longer-term effects of dietary alteration of fatty acid ratios were examined by measuring adipose tissue concentrations of fatty acids. Fully 25% of US zoo rhinos (n=20) were seen to be deficient in linoleic acid, and >50% of the samples contained undetectable levels of alpha-linolenic acid (Dierenfeld and Frank, 1998). Currently, plasma fatty acid concentrations are being summarized in US and European zoo rhinos compared with free-ranging animals, and a publication should be forthcoming.

It is suggested to limit foods high in PUFAs fed to rhinos, unless diets contain adequate antioxidant concentrations. Various feedstuffs contain antioxidant activity, including polyphenolics (tannins), vitamin E, and possibly even foods high in tyrosine (see above). Concentrates, in general, will have higher levels of PUFAs compared with forages.

### **Ongoing Captive Nutrition Studies**

A number of studies continue with captive rhinos; expect future updates. Saint Louis Zoo (n=1) has initiated feeding the White Oak Browsing Rhino pellet to add to the 3 other facilities in which this product was tested. Busch Gardens has initiated a diet study with 3 black rhinos fed a diet containing increased levels of soluble carbohydrates. A feeding trials examining seasonal intake and digestion in Sumatran rhino (n=1) is underway at Los Angeles Zoo. Tannin feed additives are being tested in relation to Fe balance in European and US zoos, but no progress updates have been submitted to the TAG. Finally, there is still interest in compiling a rhino browse and diet database (Internet-accessible); this is a topic for further input of AAZK and rhino managers.

### **Nutrition Field Studies**

Stephane Helary, a PhD student at the University of Witwatersrand, South Africa, is evaluating feeding ecology in multiple sites in Namibia and South Africa. He will be examining feed plants as well as fecal samples, quantifying Fe, tannins, minerals, and natural chelators in the diet of browsing black rhinos in differing ecosystems throughout different seasons. This project was initially funded by IRF/SOS Rhino, and should provide excellent information that can be used to assess diet optimality for this species.

### **Priority Target Areas**



Several topic areas outlined in the International Rhino Foundation 2005 Request for Proposals included possible Nutrition components:

- Dietary/Nutritional links with disease or disease susceptibility
- Dietary links with reproduction
- Nutritional links with male sex skew in rhinos

Studies encompassing these topics should provide essential information for continued understanding and improved intensive management of the rhinoceros species. Nutrition, in these cases, provides a solid foundation for enhanced conservation activities.

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