Development of a Pelleted Diet for Browsing Rhino Species Based on Native Plant Composition

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Rationale

The black rhinoceros (*Diceros bicornis*) is a browser in its native environment. In captive situations, acquiring sufficient fresh browse to meet the animal's needs is difficult or seasonally impossible at many zoological institutions. The species has been plagued with a plethora of health disorders thought to have dietary and immune system implications (Lung et al., 1998; Miller, 1999). Captive diets have variously been suggested to be excessively digestible (Atkinson et al., 1997), and imbalanced in minerals (see Paglia et al., 2000), vitamins (Dierenfeld et al., 1988; Ghebremeskel et al., 1988); fatty acids (Grant et al., 2001; Suedmeyer and Dierenfeld, 1998; Dierenfeld and Frank, 1998), protein, and carbohydrate constituents (Dierenfeld et al., 1995). A wood-based pellet was formulated and manufactured in an attempt to meet some of the nutritional and physiological needs identified for this browsing specialist.

Product Specifications

Initial nutrient specifications for the product were based on a combination of data summarized from the literature on black rhinoceros, incorporating estimated nutritional requirements derived through animal responses from blood nutrient parameters as well as known dietary browse composition. The initial test product was created in 1997 using aspen (*Populus* sp.) as the fiber source, but pelleting qualities were poor. A substitute wood source comprising pressed white oak (*Quercus alba*) was selected as the fiber source following toxicological and chemical evaluations. Nutrient specifications of the product are found in Table 1.

Facilities and Animal Data

The pellet was tested at 3 zoological institutions using five adult animals: White Oak Conservation Center (n=2), Fossil Rim Wildlife Center (n=1), and Denver Zoological Gardens (n=2). Five-day intake trials were designed to measure total intake and fecal output. Original diets (mixed grass/legume hay, low-fiber pellets, produce, fresh browse [at White Oak and Fossil Rim only], salt and/other supplements) consumed by animals at all facilities were used as the control, and were not altered during the study. The test diet was gradually introduced by replacing original pellets with the wood-fiber pellets over a 10-day adaptation period, with no other change in dietary husbandry. Total amount of pellets to be fed was estimated at 0.75% of body mass (for 910 kg animal, approximately 7 kg of pellets). Animals were fed the new pelleted diet for at least 30 days prior to recording intake and output, and one-kg samples of dry feeds and fecal samples were collected for chemical analysis. A third trial examined effects of fatty acid alteration in the diet, by supplementing test diets with 450 ml canola oil daily, again fed for a 30-day period prior to animal and diet sampling.

Table 1. Specifications and composition of wood-based browsing rhino pellet. All nutrients (except water) on a dry matter basis (mean ± s.d.).

Nutrient	Specs for Pellet	Nutrena Rhino Pellet (n=3)
DM, %		91.21 ± 0.81
Crude Protein, %	13.0	15.8 ± 1.04
Crude Fat, %	8.0	7.5 ± 2.0
C 18:2	0.5	3.4
C 18:3	5.0	0.5
NDF, %	38.5	38.9 ± 1.8
ADF, %	26.0	26.8 ± 0.5
Lignin, %		6.5 ± 0.7
Water Sol CHO, %		15.6
Vitamin A, IU/kg	7600	7200 ± 2433
Vitamin E, IU/kg	150	132 ± 10
Ash, %		6.8 ± 0.7
Macrominerals		
Ca,%	1.1	1.2 ± 0.1
Mg, %	0.2	0.2 ± 0.03
Na, %	0.3	0.2 ± 0.02
P, %	0.6	0.6 ± 0.1
Trace elements		
Cu, mg/kg	50	57 ± 4
Fe, mg/kg	100	466 ± 54
Mn, mg/kg	120	141 ± 16
Zn, mg/kg	120	170 ± 38
Se, mg/kg	0.3	0.3

Blood samples were taken following each 30-day treatment period. Plasma concentrations of fat soluble vitamins A and E (retinol and ?-tocopherol, respectively), minerals, and fatty acids were evaluated as measures of physiological nutritional status.

Intake and Digestion

No palatability problems were reported at any of the facilities, with the animals readily consuming the product (complete switchover in <2 weeks). One rhino from White Oak consumed the wood-based pellet initially, but went off feed during the trial apparently unrelated to diet or identifiable health factors. The same behavioral pattern was also seen with other feeds for this iudividual. The pellet made up 33 to 50% of dry matter intake during feeding trials at the various institutions Results indicate that the wood-based pellet was less digestible (53%) compared with a lower-fiber, grain-based diet (67%). Diet digestibility when the wood-based pellet was included resembled that measured in free-ranging black rhinos, between 30 and 50% (Atkinson et al., 1997). Lower dietary digestibility when oil was added as a treatment (46%) may have been due to a faster passage of ingesta; however, passage rate was not monitored in this study.

Physiological Measures of Nutrient Status

Vitamin analyses of blood collected from test animals indicated an overall rise in a-tocopherol levels (vitamin E) with the new pellet, and especially when canola oil was added to the wood-based pellet treatment (Table 2). This supports data from the literature which suggests a need for added dietary fat to increase fat soluble vitamin absorption. Vitamin E levels for all rhinos were within previously reported ranges for the species with all dietary treatments (Clauss et al, in press). There were no substantive changes in retinol (vitamin A) levels in any animals, and values were within normals expected for this species.

With the exception of phosphorus, plasma mineral analyses (Table 3) were within ranges expected for black rhinoceros (Dierenfeld et al, in press). Although data were incomplete for this sample set (results were limited to Fossil Rim values from their single animal receiving for both treatments, and to a single animal receiving the pellets plus oil treatment from White Oak), the pellet appeared to normalize circulating to expected range for equids (27-50 µg/ml). Since hypophosphatemia has been reported as problematic in

Table 2. Plasma vitamin concentrations for black rhinos fed mixed grass:legume hay and low fiber grain-based pellets (control), the same hay and wood-based pellets, (Treatment 1) and hay, wood-based pellets, and a canola oil supplement (Treatment 2).

	Diet Treatment		
	Control (n=3)	1 (n=5	2 (n=5)
a- tocopherol, µg/ml (vitamin E)	0.79 ±0.49	1.11 ±0.37	1.45 ±0.57
Retinol, µg/ml (vitamin A)	0.06 ±0.01	0.07 ±0.02	0.06 ±0.01

Table 3. Plasma mineral concentrations for black rhinos fed mixed grass:legume hay and low fiber grain-based pellets (control), the same hay and wood-based pellets, (Treatment 2) and hay, wood-based pellets, and a canola oil supplement (Treatment 3). All values µg/ml.

	Diet Treatment		
	Reported Range[1] (n=42)	2 (n=1)	3 (n=2)
Ca	114 — 127	115	119
Cu	0.6 – 2.1	1.5	1.8
Cr	<0.1	<0.1	<0.1
Fe	0.7 - 2.7	1.9	2.4
Mg	19.5 – 27.0	24.7	22.7
P	39.1 – 40.9	50.9	51.2
Zn	0.3 - 1.7	0.9	1.0

[1]Summarized from published data on black rhinos, Dierenfeld et al. 2001

captive rhinos, this may be a desired result but requires further investigation. The pellet also contained a higher iron concentration than desired, resulting in a calculated increased dietary iron intake compared with control diets at Denver (3.7 kg/year compared with 1.9 kg/year); iron uptake and mineral balance, however, was not measured in this study.

Results of plasma fatty acid concentrations (Table 4) demonstrate large animal variability, though the trends for the fatty acids are the same between animals. Second, for most fatty acids, values collected from Fossil Rim and White Oak animals were lower than comparable data from the Denver Zoo animals (data not shown). Third, and most importantly, there seemed to be a decrease in linoleic acid (C18:2) levels relative to linolenic acid (C18:3) for four animals with added dietary supplementation of canola oil. This supports our original hypothesis that canola oil could be a useful dietary source of linolenic acid, and possibly alter the high linoleic:linolenic acid ratio documented in diets of zoo black rhinos (Suedmeyer and Dierenfeld, 1998; Dierenfeld and Frank, 1998). However, canola was not optimal in altering fatty acid ratios, since it still contains more linoleic compared with linolenic acid, and other alternatives will be explored.

General Observations

Observations made at the Denver Zoo (and other facilities) have documented that when browse is consumed by black rhinos, urine color changes to a rust-orange color. Initially when the wood-based pellet was fed, this "phenomena" also occurred, with no browse being introduced into the diet. This observation was not reported at the other institutions, which fed browse as a component of the control diet. Presumably this is due to the presence of condensed tannins, although tannin analysis of the formulated pellets showed them to contain <0.1% tannic acid equivalents (total phenolics), considerably lower than reported previously in leaves of this species (5 to 10%). Also, anecdotally at the Denver Zoo, the skin condition of the animals was improved, and an alteration in animal behavior was noted – the animals seemed "more content".

Table 4. Plasma fatty acid concentrations for black rhinos fed mixed grass:legume hay and low fiber grain-based pellets (control), the same hay and wood-based pellets, (Treatment 1) and hay, wood-based pellets, and a canola oil supplement (Treatment 2). Mean ± s.d.

Fatty Acid	Diet Treatment Control (n=2)	1 (n=4)	2 (n=4)
12:0	0.6 ± 0.3	0.4 ± 0.2	0.2±0.1
14:0	2.7 ± 1.2	1.4 ± 0.8	1.2 ± 0.6
15:0	0.5 ± 0.03	0.4 ± 0.2	0.3 ± 0.07
16:0	15.9 ± 2.0	14.0 ± 1.4	15.4 ± 1.5
16:1	1.2 ± 0.3	0.9 ± 0.2	1.3 ± 0.2
17:0	0.9 ± 0.1	0.7 ± 0.2	0.6 ± 0.2
17:1	1.0 ± 0.1	0.5 ± 0.4	0.6 ± 0.3
18:0	10.3 ± 0.5	8.8 ± 1.1	8.3 ± 1.3
18:1	13.8 ± 2.2	14.2 ± 1.9	18.4 ± 2.1
18:2	35.5 ± 0.7	46.0 ± 7.6	41.1 ± 7.3
18:3	1.2 ± 0.02	1.0 ± 0.5	0.8 ± 0.5
20:0	0.9 ± 0.1	0.6 ± 0.2	0.6 ± 0.3
20:4	0.8 ± 0.3	0.8 ± 0.6	1.3 ± 0.9
20:5	0.2 ± 0.2	0.1 ± 0.1	0.3 ± 0.2
22:0	0.9 ± 0.2	0.8 ± 0.1	0.6 ± 0.1
22:2	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
24:0	1.3 ± 0.1	0.7 ± 0.2	0.7 ± 0.3
24:1	0.8 ± 0.1	1.2 ± 0.5	1.3 ± 0.4
unknown	11.4 ± 5.6	6.0 ± 3.2	6.1 ± 3.2

Product Improvements

Some modifications to the mineral and fatty acid content of the original formulation were suggested from these data, including the use of purer grade mineral sources, removal of iron from the trace mineral premix, and the addition of flax oil as the primary source of balanced fatty acids. This wood-based pellet has been further developed and marketed for feeding the North American zoo black rhinoceros population, with a portion of all sales directed to the International Rhino Foundation to support further conservation efforts. Controlled intake, digestibility, and nutritional studies on a larger study group are currently underway.

Conclusions

1) The chemical composition of native browses provided reasonable nutrient guidelines for the development of palatable wood-based pellets fed to black rhinoceros in 3 zoological institutions.

2) White oak (*Quercus alba*) was suitable as a fiber source for this pellet, both chemically and physically.

3) Overall diet digestibility decreased when the wood-based fiber pellet was consumed by black rhinoceros, to a level considered appropriate for this species (approximately 50% of DM).

4) Fat-soluble vitamin status appeared to increase or showed no change on the test diets.Circulating vitamin E concentration increased with the inclusion of canola oil in the diet. Overall, the levels of these nutrients in the test pellet appear adequate for black rhinoceros in captivity.

5) The mineral content of the manufactured pellet was not optimal in the final product compared with desired specifications; however, physiological mineral status in the rhinos could not be evaluated from the limited data available. Iron content, in particular, will be decreased in future pellet formulations.

6) Plasma fatty acid ratios, an indicator of short-time fatty acid status, appeared to be altered favorably in black rhinoceros by the addition of canola oil to the diet. Flax oil, with an even higher ratio of C18:3 to C18:2 fatty acids, was selected as an ingredient for incorporation into future pellet formulations to meet fatty acid needs of the browsing rhinos.

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Red Cell Metabolism in the Black Rhinoceros: Relevance to Haemolytic Disease

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Captive black rhinoceros populations in the USA have been afflicted with a severe haemolytic anaemia syndrome, together with a leukoencephalopathy and other disorders suggestive of a free radical pathologic basis, and this has been the subject of intensive metabolic investigation for some years in our laboratories. The black rhinoceros (*Diceros bicornis*) shows a number of striking differences in its normal red cell biochemistry compared with humans: enzyme levels are often grossly different, ATP levels are 1/50th that of humans, and they contain very high levels of free tyrosine in their red cells (but not in plasma). On exposure to oxidative stress some tyrosine is converted transitorily to dityrosine, a substance never previously described in free form in cells, with an inverse relationship to glutathione levels. Human red blood cells incubated under the same conditions show no sign of dityrosine production.

Tyrosine is known to be a substrate for oxidative reactions, and has been implicated in contributing to defence against oxidative damage in seminal plasma. Experiments will be described which suggest that that tyrosine, together with some purine metabolites, are acting as an additional defence mechanism against reactive oxygen intermediates in red cells with marginal protective mechanisms. Oxygen radical absorbance (ORAC) assays, together with red cell tyrosine and purine levels, are currently being compared between in situ rhinoceroses in South Africa and captive (ex situ) individuals in Europe and the USA,. The integration of these in vitro and in vivo analyses should reveal insights and mechanisms exploitable for the development of preventative or therapeutic measures against haemolytic and other free radical induced disorders in these populations.

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