Counting Rhinos From Dung: A Method to Estimate the Minimum Number of Animals Present in a Protected Area Using Microsatellite DNA

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We report a DNA-based method to estimate the minimum number of individuals in a population of black rhinoceros, *Diceros bicornis*, in a reserve in southern Tanzania. The number of individuals present in this population could not be determined by conventional means for a number of reasons. In this pilot study, total genomic DNA was extracted from dung samples collected in the reserve and polymorphic microsatellite DNA loci were amplified using the Polymerase Chain Reaction. Although very low amounts of DNA were extracted, positive amplification products were obtained from 60% of the dung samples. For the remaining samples, plant inhibitors co-extracted with the rhinoceros DNA prevented the amplification of the microsatellite loci. Nine unique genotypes were observed using polymorphic black rhinoceros specific primers. Preliminary results suggest that, although the technique is not as yet reproducible, it provides the basis for non-invasive and cost effective sampling of rare and endangered animals in the wild.

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

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The black rhinoceros (*Diceros bicornis*) is a browser in its native environment. Acquiring sufficient browse to meet the animal's feeding needs is difficult or impossible at most zoological institutions, and captive diets have typically been based upon concentrate and forage rations originally designed for domestic species. Such diets appear excessively digestible, and are often quite disparate in mineral, vitamin, fatty acid, protein, and carbohydrate constituents relative to native plants consumed. In an attempt to address some of the nutritional and physiologic needs of this browsing specialist, a wood-based (white oak, *Quercus alba*) pellet was formulated using the chemical composition of native browses as nutrient guidelines. Palatability studies were conducted at three facilities (n=5 black rhinos); intake and digestion information was collected at two locations (n=3 black rhinos). Blood samples were obtained to examine nutrient status pre- and post-feeding trials (n=5 black rhinos). All animals readily consumed the new product in addition to mixed legume/grass hay. Diet digestibility dropped from approximately 65% to 50%, a level similar to that measured in free-ranging rhinos eating native browses. Vitamin analyses indicated an overall rise in a-tocopherol levels (vitamin E) with the new pellet. There were no substantive changes in retinol (vitamin A) levels in any animals, and values for both vitamins were within normal ranges expected. Plasma fatty acid ratios, as indicators of recent fatty acid intake, appeared to be altered favorably by the addition of canola oil supplementation to the diet treatment and better duplicate those found in free-ranging rhinos. Modifications to the mineral and fatty acid content of the initial formulation were suggested from these preliminary data, and the pellet was further refined for commercial production. More extensive intake, digestibility, and investigations of physiological status are currently underway on a larger study group within the North American zoo black rhinoceros population.

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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