ESSENTIAL FATTY ACIDS, TOTAL LIPID, AND CONDENSED TANNIN IN THE DIET OF CAPTIVE BLACK RHINOCEROSES (*DICEROS BICORNIS*) IN NORTH AMERICA AND IN BROWSES NATIVE TO ZIMBABWE, AFRICA

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ABSTRACT

Necrolytic migratory erythema (NME) is a disease seen in captive black rhinoceroses in North America which manifests itself by the appearance of waxing and waning mucocutaneous ulcers which sometimes cover up to 70% of the affected captive black rhinoceros' skin. NME has been speculated to be caused by deficiency of essential amino acids, fatty acids, and/or complex sugars.

The objectives of the current work were to explore the possible connection of NME to essential fatty acid deficiency in the diet of captive black rhinoceroses by comparing the amounts of total lipids and essential fatty acids found in three variant diets (a composite diet of what is currently offered to captive black rhinos in North America, a fresh African browse based diet,f both types of diets was also comparable. Linoleic acid declined by an average of 40% after 140 days and a-linolenic acid declined by an average of 90% after 140 days of storage in a dry state. Gamma linolenic acid was not found in these samples.

This work identified a massive imbalance in the ratio of ingested linoleic acid to a-linolenic acid in the captive black rhinoceros in North America as opposed to fresh-browse-only-fed black rhinos.

BIOGRAPHICAL SKETCH

Jacqualine B. Wright was born in Rochester, New York on April 13, 1968. She was raised in the suburb of East Irondequoit where she took to the woods and swimming whenever possible. Her interest in animals began at a young age when she was introduced to the wonderful world of amphibians by her maternal grandfather and to the forest by her paternal grandfather. She received her B.S. in Biochemistry from Texas A&M University in 1990. After graduating, she work as a technician in a soil, water, and air chemistry lab in College Station, Texas. She then escaped back to New York where she worked for a year as a technician in a physical organic dye chemistry lab at Eastman Kodak Company in Rochester, NY. Then she moved on to better things by gaining a job as a pesticide chemist in the Department of Food Science of Cornell University at the New York State Agricultural Experiment Station in Geneva, NY. While in Geneva, she taught swimming lessons at the local YMCA and joined the Canandaigua Sawbellies Masters swim team. She began taking courses in the Department of Animal Science at Cornell University in the spring of 1994 as a Cornell employee. She was accepted into the Masters program in the Department of Animal Science at Cornell University in January of 1995. She still maintains her interest in amphibians.

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TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF FIGURES

PAGE

Figure and a fresh North American browse based diet) offered to black rhinoceroses. In addition, a survey of the diets currently fed to captive black rhinoceroses in North America was made in an effort to more clearly depict their nutrition status. Finally, a colorimetric analysis of condensed tannins in both wild and captive rhinoceros diets was conducted.

Total lipid, linoleic acid and a-linolenic acid content were determined for samples collected from North American black rhino holding facilities, Zimbabwe, Africa, and New York State. All samples were air-dried and ground to a 2 mm mesh. Methyl esters of the fatty acids (FAMEs) of interest were extracted from the samples using a variation of a micro-extraction/methylation method from Browse, et al. (1986) that consisted of heating approximately 30-60 mg of sample at 80°C in 1 N methanolic HCl, 5% 2,2-dimethoxypropane for an hour, followed by extraction of FAMEs by 1 mL hexane with 1 mL of 1% NaCl solution. Two hundred µL of the hexane phase of each sample was then injected onto a gas chromatograph equipped with a mass spectrometer detector (GC/MS). A fused silica capillary column with a biscyanopropyl polysiloxane film was installed on the GC/MS to separate the FAMEs by retention time (Rt). Identification of the FAMEs was confirmed by comparison of peaks with Rt matching those of interest to a spectral library generated by the injection of known standards. An internal standard of 200 µL heptadecanoic acid was added to each sample prior to digestion. A study on the degradation of linoleic acid and n-3 linolenic acid content was also conducted by measuring the essential fatty acid contents of ten North American browses at 0 days (fresh, whole sample) and again at 140 days (dried, ground sample). Total lipid content of each sample was determined using ether extraction. Condensed tannin content was rated colorimetrically on a scale of zero to three, with zero being no red pigment present and three being very dark red pigment present.

Condensed tannin content in both of the browse diets averaged 2 out of 5, while in the composite diet tannin rating was only 0.2 out of 5. Total intake of linoleic acid and a-linolenic acid by rhinos ingesting 27.7 kg/day of the composite North American diet was 78 g and 81 g. Intake of linoleic acid and a-linolenic acid by rhinos ingesting 30 kg/day of fresh African browse was 13 g and 240 g. Intake of linoleic acid and a-linolenic acid by rhinos ingesting 30 kg/day of fresh North American browse was 14 g and 100 g. Total lipid content

CHAPTER I: LITERATURE REVIEW

1.1 INTRODUCTION

The family of rhinocerotidae currently contains five species of rhinoceroses. Four of these species, *Rhinoceros unicornis*, *Rhinoceros sondaicus*, *Dicerorhinus sumatrensis*, and *Diceros bicornis*, are in grave danger of extinction (Foose, 1996). The fifth species (*Ceratotherium simum*) contains two subspecies, one of which (the southern white rhinoceros) has made a remarkable comeback in recent years while the other subspecies (the northern white rhinoceros) remains the most rare and endangered of all the rhinoceroses (Foose, 1996). As of the year 1995, approximately 85% of the world's population of all rhinoceroses had been lost, with the black rhinoceros in Africa declining the fastest, from an estimated 65,000 in 1970 to about 2,550 in 1995 (Kelly et al., 1995). Current poaching practices, hoarding of rhinoceros products, and habitat encroachment are all combining to make the future survival of the rhinocerotidae family look rather dismal.

Conservation of the black rhinoceros (*Diceros bicornis*) is an important problem to which the American Zoo and Aquarium Association (AZA) has devoted a large amount of effort. The black rhinoceros is threatened primarily by poachers and to a lesser extent from habitat encroachment. The AZA, through its Species Survival Plans (SSPs), has developed captive programs for the black rhinoceros and other endangered animals to help them survive the current crisis. Several organizations around the world are making efforts towards preserving the safety and future of the rhinoceroses. The purpose of the SSPs is to provide guidelines for the successful maintenance of captive populations of rhinoceroses in order to sustain their populations in numbers high enough to prevent or delay the extinction threatening them.

The SSPs have been successful in their efforts towards the healthy and successful captive maintenance of the white rhinoceros (*Ceratotherium simum*). In fact, the current plan calls for a reduction in the total number of white rhinoceroses (Foose and Miller, 1994), but not its other African relative, the black rhinoceros (*Diceros bicornis*). The captive black rhinoceros is prone to several unusual diseases not found in captive white rhinoceroses nor in wild black rhinoceroses (Miller, 1994).

The diseases specific to the captive black rhinoceros population include hemolytic anemia (Miller & Boever, 1982; Miller, 1993a; Paglia and Miller, 1993; Paglia, 1993), fungal pneumonia (Miller, 1993b; Miller, 1994; Miller, 1996), mucosal and cutaneous ulcerative syndrome (Ott et al. 1982; Munson, 1993) now more accurately referred to as necrolytic migratory erythema (NME) (Munson et al., In press) or superficial necrolytic dermatitis (SND) (Miller, 1995), encephalomalacia (Miller et al., 1990), and tissue accumulation of iron (Montali, 1993).

The following review will discuss the differences between the two African rhinoceros species, *Diceros bicornis* and *Ceratotherium simum*, that may be important in explaining the differing levels of success the two species

have had in captivity in North America (Miller, 1993b). Nutritional effects of tannins and the differences between condensed and hydrolysable tannins will be discussed briefly. This will be followed by an examination of the rare disease NME and its equivalent in the dog, superficial necrolytic dermatitis (SND), and the implications of the essential fatty acids linoleic acid and alpha linolenic acid as possible causative agents in these diseases. Other possible causes of NME and SND will be reviewed as the exact pathogenesis of this disease has not yet been determined (Masri-Fielding and Turner, 1992; Wermers et al., 1996). Possible roles of the essential fatty acids and dietary linoleic acid:alpha linolenic acid ratio in dermatopathies and other health problems will also be examined.

1.2 THE AFRICAN RHINOCEROSES

There are two species of African rhinoceroses, the black rhinoceros (*D. bicornis*) and the white rhinoceros (*C. simum*). Each of the species can be divided into two subspecies, the black rhinoceros into the Eastern (*D. b. michaeli*) and Southern (*D. b. minor*) types and the white rhinoceros into the Northern (*C. s. cottoni*) and Southern (*C. s. simum*) types. Both of these rhinoceros species have suffered huge declines in their native ranges (Guggisberg, 1966; Penny, 1988). The two species are of a similar size and color with the white rhinoceros being the larger, but have a radically different approach to feeding. These differing feeding strategies could very well be the key to why white rhinoceroses have prospered in captivity and black rhinoceroses have not (Miller, 1993b). The white rhinoceros is not white nor is the black rhinoceros black. Early explorers of Africa mistook the Afrikaans name "weit rhino" ("weit" meaning "wide" in reference to the wide lip of the white rhino) to be "white" rhino (Penny, 1988). Consequently, this lead to the misnomer of black rhino upon the subsequent discovery of a second rhino species in Africa; if it wasn't the white rhino, it must be the black!

1.2.1 THE WHITE RHINOCEROS (*CERATOTHERIUM SIMUM*)

The white rhinoceros, depicted in Figures 1.1 and 1.2, is known by the common names of the grazing rhinoceros and the square lipped rhinoceros; these names are indicative of its feeding strategy. The white rhinoceros, despite sharing the same ecological habitat (see in Figures 1.3 and 1.4) as the black rhinoceros (Guggisberg, 1966), is primarily a selective grazer (Pienaar, 1994) as shown in Figure 1.5. The wild white rhinoceros' dietary preferences are completely opposite those of the black rhinoceros (Pienaar, 1994; Goddard, 1968). The white rhinoceros will avoid stands of its preferred grasses if too many forbs are present in the grass stand (Pienaar, 1994) while the black rhino will choose browses over grasses regardless of season (Dierenfled et al., 1996).

Previously, the white rhinoceros was thought to be a model for the black rhinoceros because of their similarity in size and habitat. The white rhinoceros is almost certainly not a good model for the management of the black rhinoceros because of the radical difference in their feeding patterns (Dierenfeld et al., 1995). This difference in feeding patterns may be indicative of differences in digestive physiology between the two species (Maloiy and Clemens, 1991) that could lead to nutritional deficiencies if either one was fed a diet based on the other.

1.2.2 THE BLACK RHINOCEROS (*DICEROS BICORNIS*)

1.2.2.1 Dietary patterns and digestive physiology of the wild black rhinoceros.

It is well documented that the black rhinoceros is primarily a strict browser of a very large number of species of plants (Goddard, 1968; Goddard, 1970; Mukinga, 1977; Loutit et al. 1987; Ghebremeskel et al., 1991; Miller, 1993; Emslie and Adkock, 1994; Oloo et al., 1994; Maddock et al. 1994; Dierenfeld et al. 1995). Due to the number of species of plants it eats, the diet of wild black rhinoceroses is not easily defined beyond the fact that it heavily prefers browses over grasses (Dierenfeld et al., 1995). Its diet depends heavily on geography and seasonality (Goddard, 1968; Oloo et al. 1994; Duncan, 1994). The black rhinoceros is also able to tolerate a diet consisting of more fibrous material than other large herbivores (Oloo et al., 1994). This ability to thrive on highly fibrous, woody materials and its avoidance of grasses may be key to its current nutritional problems in captivity in North America. Herbivore browsers in particular are known to be prone to maladies due in part or whole to nutritional imbalances or deficiencies in captivity (Ghebremeskel et al., 1988). At the Port Lympne Zoo in the United Kingdom, black rhinoceroses which have been fed primarily browses indigenous to the zoo's area have not experienced the unusual diseases seen in North American captive black rhinoceroses (Furley, 1993) nor did three black rhinoceroses at the Dvur Kralove Zoo in Czechoslovakia that were fed a diet of ZOO I granulated concentrate, oat grain, and meadow hay (Spala and Hradecky, 1993).

Figure 1.1 The Square-lipped Rhinoceros (*Ceratotherium simum*) in Matobo National Park, near Bulawayo, Zimbabwe, Africa. Note the ideal lip-shape for grazing.

Figure 1.2 The Square-lipped Rhinoceros (*Ceratotherium simum*) in Matobo National Park, near Bulawayo, Zimbabwe, Africa. Note the ideal lip-shape for grazing.

Figure 1.3 An example of the habitat shared by both *Diceros bicornis* and *Ceratotherium simum* in Matobo National Park, Zimbabwe, Africa. Note the combination of brush and grassland which makes the habitat suitable for both species.

Figure 1.4 Another example of the habitat shared by both *Diceros bicornis* and *Ceratotherium simum* in Matobo National Park, Zimbabwe, Africa. Note the combination of brush and grassland which makes the habitat suitable for both species.

Figure 1.5 *Ceratotherium simum* grazing high grass in Matobo Park, Zimbabwe, Africa. White rhinos much prefer grasses to browses and will avoid small bushes even in patches of grass such as this.

Figure 1.6 *Diceros bicornis* in the Chippengali Wildlife Orphanage near Bulawayo, Zimbabwe. *Diceros bicornis* has a prehensile lip suited to browsing. It is rarely seen in the wild anymore due to poaching.

Figure 1.7 The gastrointestinal tract of *Diceros bicornis* adapted from Clemens and Maloiy, 1982.

The black rhinoceros' prehensile upper lip, shown in Figure 1.6, is well suited to its diet of woody and/or succulent plants (Dierenfeld et al., 1995). The black rhinoceros' lip and eating habits are responsible for its other common names of browsing rhinoceros and hooked lipped rhinoceros. The stomach of the black rhinoceros is fairly simple, being generally noncompartmentalized (Clemens and Maloiy, 1982). As seen in Figure 1.7, the black rhinoceros has a large, sacculated caecum (Clemens and Maloiy, 1982) and a large intestine that structurally most closely resembles that of the *Perissodactyla* (i.e. the horse, pony, and donkey) (Stevens, 1977). Despite this similarity, the black rhinoceros is distinctly a browser, while the other equids are grazers, and a diet formulated for a black rhinoceros based on an equid diet (horse pellets, grass hay, and mineral supplements) could be a serious error (Ghebremeskel et al., 1988). These digestive physiological traits would suggest that the black rhinoceros has a dietary strategy of high intake and high passage rate and would most likely prefer lower quality, more fibrous food sources such as browse over high energy concentrates (Van Soest, 1994), primarily because it ingests the entire branch; it does not selectively eat only the leaves. A browser that selectively eats only leaves, leaving the twigs is actually receiving a high quality diet (Van Soest, 1994).

1.2.2.2 Diseases of the captive black rhinoceros. In captivity in North America, the black rhinoceros is prone to several diseases not noted in the wild (Miller, 1994): hemolytic anemia, fungal pneumonia, NME, encephalomalacia, and tissue accumulation of iron. Hemolytic anemia, NME, fungal pneumonia, and tissue accumulation of iron all have direct and/or indirect ties to nutritional factors (Kock and Garnier, 1993; Miller, 1993b; Miller, 1994) among others as causative agents. Captive black rhinoceroses are also subject to progressive loss of vitamin E which may be a factor in red blood cell instability (Dierenfeld et al., 1988; Ghebremeskel et al., 1988). It is also of note that catalase deficiency is characteristic of the black rhinoceros (Paglia, 1993; Paglia and Miller, 1993) and that Takahara's disease (acatalasemia and hypocatalasemia {Takahara, 1971}) is associated with mucocutaneous ulcerations similar to those seen in black rhinoceroses afflicted with NME. NME in captive black rhinoceroses is the focus of this work.

1.2.2.3 NME in the black rhinoceros. Figures 1.5 through 1.7 display the affliction seen in fifty percent of the United States population of black rhinoceroses. These black rhinos have been affected by a cutaneous and oral mucosal disease characterized by waxing and waning cutaneous lesions that begin as plaques which ultimately may result in bullae or ulcers with the same clinical patterns and histopathology of NME and SND seen in other species (Munson et al., In press). It has been suggested that NME in the captive black rhinoceros is an epidermal response to many metabolic disorders (Munson et al., In press). No pathogens have been associated with the ulcers except as secondary infection (Munson, 1993; Miller, 1995; Munson et al., In press). Given the captive black rhinoceros' history of disease and the urgency of its

situation, its possible nutritional problems are being approached from all angles in the hopes that the causes can be identified or, if not, some possible causes can be eliminated. It has been suggested that essential fatty acid deficiency might be a factor in NME in the

Figure 1.8 NME-like lesions on the pressure points of a captive black rhinoceros. Photo courtesy of R.E. Miller, St. Louis Zoo.

Figure 1.9 Gross skin ulcerations on a black rhinoceros displaying signs of NME. Photo courtesy of R.E. Miller, St. Louis Zoo.

Figure 1.10 Oral NME-like lesions on a black rhinoceros. Photo courtesy of R.E. Miller, St. Louis Zoo.

captive black rhinoceros (Dierenfeld, 1995; Munson et al., In press). Long chain fatty acid deficiency has not been produced in the horse family, relatives of the black rhinoceros (NRC, 1978). An excellent article on NME in the United States population of black rhinoceroses is currently in press by Munson et al. Dermatological problems in the black rhinoceros are not limited to the North American population with at least three black rhinoceroses in the United Kingdom with chronic ulcerative dermatitis (Kock and Garnier, 1993) and two in Australia with ulcerative skin eruptions possibly linked to liver failure (Kelly et al., 1995).

1.3 TANNINS

Another aspect to consider is tannin content. Most observations on tannins have been in the light of possible avoidance of them by animals and insects in the diet (Harbourne, 1982; Cheeke and Shull, 1985) due to their antinutritional effects. Condensed tannins have known protein precipitating effects (Van Soest, 1994) and act as multidentate ligands that can inhibit the activity of important digestive enzymes including amylases, lipases, and trypsin in vitro (Griffiths, 1991). Hydrolysable tannins are not thought to have as much of an antinutritional influence in large herbivore nutrition as condensed tannins do because of the effect of condensed tannins upon the microbial fermentation of plant cell walls that provides energy to the animal (Cooper and Owen-Smith, 1985). Hydrolysable tannins generally are hydrolyzed from their substrate during digestion which decreases their potential negative influence. Soluble or

hydrolysable tannins have a polyhydric alcohol core usually attached to one of two acids, gallic or hexahydroxydiphenic, which are readily hydrolyzed by acids or enzymes to yield carbohydrate and phenolic acid (Griffiths, 1991). Condensed tannins or proanthocyanidins are complex oligimeric derivatives of the flavan-3-ols and flavan-3,4-ols (Griffiths, 1989). These complex, large molecules do not readily release substrate once bound (Van Soest, 1994). It is not known if tannins have positive nutritional effects. Figures 1.11 and 1.12 show general structures for hydrolysable and condensed tannins.

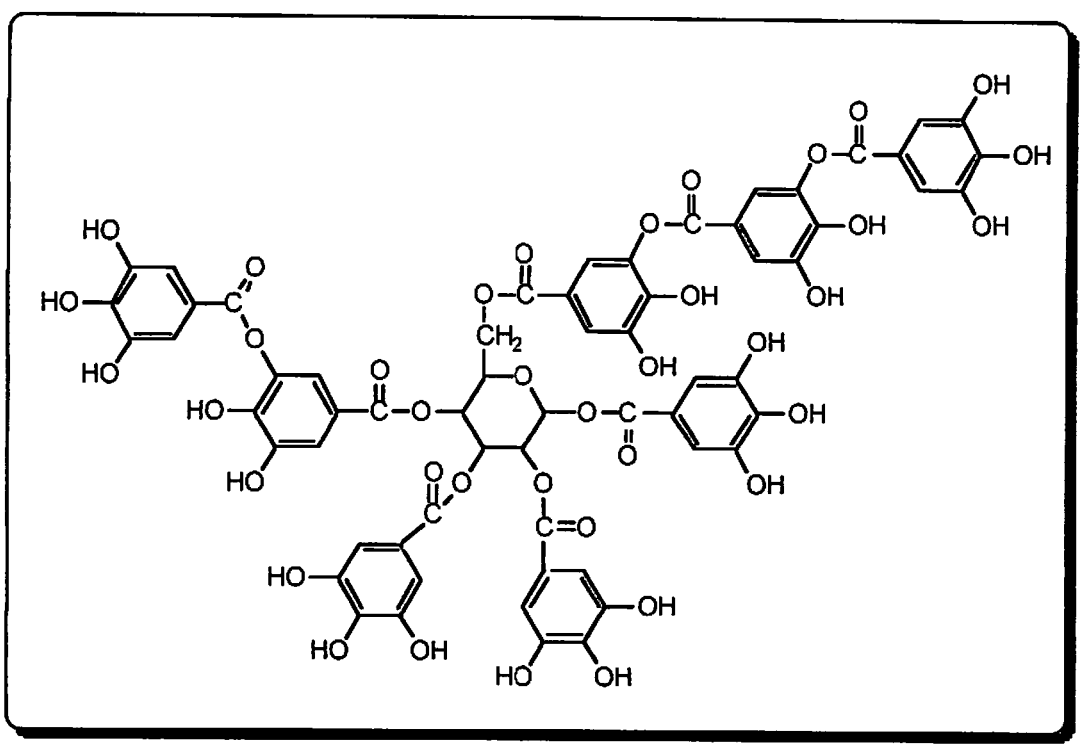

Figure 1.11 The structure of a typical hydrolysable tannin (Griffiths, 1991).

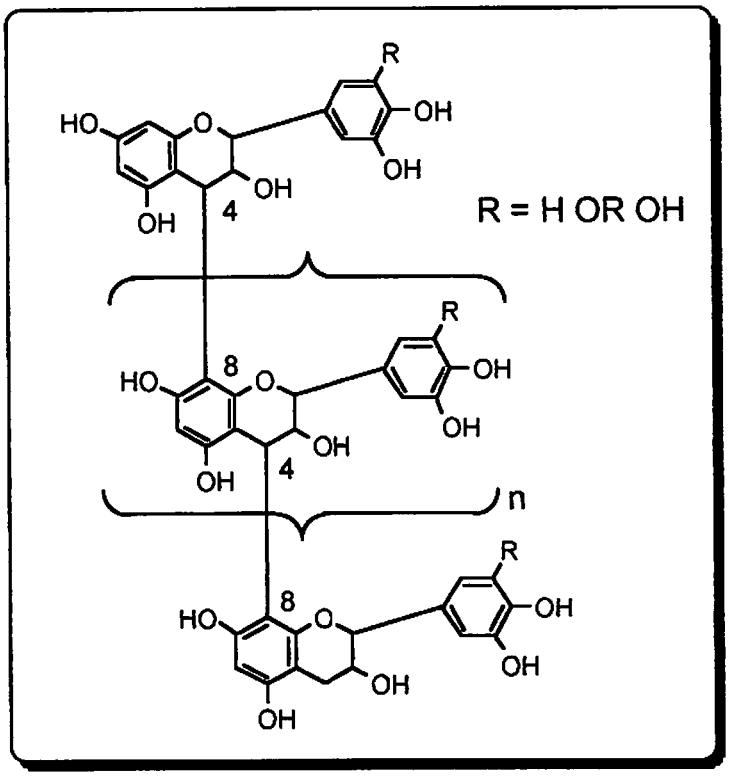

Figure 1.12 The structure of a typical condensed tannin (Griffiths, 1991).

1.4 NECROLYTIC MIGRATORY ERYTHEMA (NME) AND SUPERFICIAL NECROLYTIC DERMATITIS (SND)

Necrolytic migratory erythema and superficial necrolytic dermatitis (also referred to as canine diabetic dermatosis or ulcerative dermatosis seen in diabetic dogs {Walton et al., 1986; Turnwald et al., 1989}) are two rare skin diseases of relatively unknown etiology (Marinkovich et al., 1995; Wermers et al., 1996; Nyland et al., 1996). NME has been diagnosed in man, while SND is seen in dogs. A similar skin condition has not been identified in other species except for the recent diagnosis of a similar mucocutaneous ulcerative syndrome in the North American captive black rhinoceros (Munson, 1993).

1.4.1 SYMPTOMS OF NME And SND

NME is a rare, but well documented (Becker et al., 1942; Doyle et al., 1979; Walton et al., 1986; Turnwald et al., 1989; Blackford et al., 1991; Kasper and McMurray, 1991; Thorisdottir et al., 1994; Marinkovich et al., 1996; Nyland et al., 1996; Wermers et al., 1996), skin disease in man normally associated with glucagon-secreting alpha-cell neoplasms of pancreatic islet cells (Marinkovich et al., 1996) also referred to as the glucagonoma syndrome. This dermatosis involves well marginated, erythematous lesions which progress to erosion, crusting, and scaling due to superficial necrosis but showing some

healing towards the center; found typically in the trunk, perineum, lower extremities, and perioral area (Doyle et al., 1979; Walton et al., 1986; Thorisdottir et al., 1994). SND is the canine equivalent to NME (Walton et al., 1986; Turnwald et al., 1989; Kasper and McMurray, 1991; Nyland et al., 1996). Cases of NME and SND have been seen not involving glucagon-secreting pancreatic islet cell neoplasms (Doyle et al., 1979; Blackford et al., 1991; Kasper and McMurray, 1991; Masri-Fielding and Turner, 1992; Thorisdottir et al., 1994; Marinkovich et al., 1995;). Such cases are sometimes referred to as pseudoglucagonoma syndrome or canine hepatocutaneous syndrome (Kasper and McMurray, 1991). Several theories have been proposed for these two versions of NME and SND.

1.4.2 CAUSES AND TREATMENT OF NME/SND

NME and SND are difficult to pinpoint treatments for, even when apparent successful treatment is observed, due to their inconsistent nature (Kasper and McMurray, 1991). The lesions involved have been known to spontaneously resolve without treatment (Munson, 1993), further confounding the diagnosis of the underlying causes of the disease. Theorized causes for NME and SND have ranged from malnutrition with deficiencies of essential fatty acids, amino acids, vitamins, and zinc (Thorisdottir et al., 1994), essential fatty acid deficiency alone (Walton et al., 1986; Blackford et al., 1991; Wermers et al., 1996), malabsorption syndrome (Walton et al., 1986; Thorisdottir et al., 1994), zinc deficiency syndrome (Walton et al., 1986; Hansen, 1992; Nyland et al., 1996; Wermers et al., 1996), n-3 marine essential fatty acids (Delaney and Uff, 1990), hypoaminoacidemia (Walton et al., 1986; Turnwald et al., 1989; Nyland et al., 1996; Wermers et al., 1996), hepatic cirrhosis or impairment (Doyle et al., 1979; Turnwald et al., 1989; Kasper and McMurry, 1991; Marinkovich et al., 1995; Nyland et al., 1996; Wermers et al., 1996), hypoalbuminemia (Marinkovich et al., 1995) excess arachidonic acid synthesis leading to inflammation and necrosis of areas of skin subjected to trauma (Doyle et al., 1979; Walton et al., 1986; Nyland et al., 1996), hyperglucagonemia, repeated trauma or friction in general, kwashiorkor, toxic epidermal necrolysis, pemphigus variants, systemic lupus erythematosus, vasculitis, candidiasis, allergic contact dermatitis, erythema multiforme, and dermatitis herpetiformis (Walton et al., 1986). In considering these theories, it must be kept in mind that strong arguments can be made both for and against hypoaminoacidemia (Goodenberger et al. 1979; Abraira et al., 1984; Walton et al., 1986; Turnwald et al., 1989; Blackford et al., 1991; Nyland et al., 1996), zinc deficiency (Turnwald et al., 1989; Blackford et al., 1991; Kasper and McMurry, 1991; Marinkovich et al., 1995), and essential fatty acid deficiency (Blackford et al., 1991; Kasper and McMurry, 1991). Given these discrepancies, all avenues for exploration of causes of NME/SND in the black rhinoceros should be taken into consideration.

1.5 DERMATITIS AND NUTRITIONAL DEFICIENCY

1.5.1 LINOLEIC ACID (N-6) AND ALPHA-LINOLENIC ACID (N-3)

The fatty acids linoleic acid and n-3 linolenic acid are the two most important polyunsaturated fatty acids (PUFA) in nutrition. The importance of PUFA was demonstrated as early as 1930 (Burr and Burr, 1930). There are two noninterchangeable groups of PUFA, the n-3 and n-6 families with linoleic acid (18:2n6) as the precursor for the n-6 family and alpha linolenic acid (18:3n3) as the precursor for the n-3 family. The essentiality of the n-6 family has been fairly well understood for some time, while that of the n-3 family is less obvious and has only been come to be defined recently (Holman and Johnson, 1981; Fayard, 1992). Linoleic acid has much greater EFA activity than n-3 linolenic acid, it has much greater growth promoting activity and it can cure EFA deficiency-caused dermatitis (Holman and Johnson, 1981). Alpha-linolenic acid deficiency is seen more readily in the function of nervous tissues (Holman and Johnson, 1981; Fayard, 1992).

Alpha-linolenic acid is a fairly ubiquitous compound which is very difficult to avoid, especially in plant materials (Hitchcock and Nichols, 1971; Zöllner, 1986). This would lead to the expectation that a deficiency of n-3 linolenic acid would be very hard to achieve. What could happen, especially in a captive animal (as seen in some fish), is a relative deficiency of n-3 PUFA if the diet was exceptionally high in n-6 PUFA versus n-3 PUFA (Fayard, 1992). High dietary ratios of (n-6)/(n-3) have been found to be harmful to human health (Fayard, 1992). Proposed mechanisms for the benefits of increased n-3 PUFA in the diet include the reduced production of n-6 derived 2-series prostaglandins and 4-series leukotrienes due to the preference of the delta-6 desaturase and elongating enzymes for n-3 linolenic acid over linoleic acid (Marshall and Johnston, 1981; Fayard, 1992).

1.5.2 GAMMA-LINOLENIC ACID (N-6), ARACHIDONIC ACID AND PROSTAGLANDINS

Cats with papulocrustous dermatitis (an inflammatory dermatosis) and children with atopic eczema both responded to dietary treatments containing high levels of n-6 linolenic acid (gamma-linolenic acid) (Harvey, 1993a; Harvey, 1993b; Shimasaki, 1995). Gamma-linolenic acid is the post delta-6 desaturase product of n-6 linoleic acid (Harvey, 1993b; Shimasaki, 1995) and is the proximal step to the formation of arachidonic acid and its metabolites (Brenner, 1981; Horrobin and Cunnane, 1981; Richard et al., 1990; Shimasaki, 1995) which has been implicated in NME (see section 1.3.1). It has been suggested that n-6 linolenic acid is effective in children with atopic eczema and cats because both are lacking significant capacity to desaturate n-6 linoleic acid (Harvey, 1993b; Shimasaki, 1995). If n-6 linolenic acid were found to be a significant component of the wild black rhinoceros' preferred browses, this could indicate a possible delta-6 desaturase inadequacy in the black rhinoceros.

The inability to desaturate n-6 linoleic acid may lead to an imbalance between the prostaglandin series PG1 and PG2 (Richard et al., 1990). The eicosanoids in the PG1 and PG3 series are believed to be primarily anti-inflammatory in nature, while those in the PG2 family and the leukotrienes are believed to be pro-inflammatory mediators (Harvey, 1993b). It has been proposed that the n-6 fatty acids are involved primarily in the synthesis of anti-inflammatory eicosanoids, but direct metabolism of these eicosanoids is not responsible for the amelioration of papulocrustous dermatitis in cats (Harvey, 1993a). It is speculated that EFA maintain cutaneous integrity through the formation of prostaglandins in the tissue (Ziboh et al., 1981).

1.5.3 ZINC DEFICIENCY

Research has shown that the wild black rhinoceros in Zimbabwe consumes a diet that may be on the borderline of adequate for consumption of zinc (Dierenfeld et al., 1995). In horses, zinc deficiency is known to cause cutaneous lesions on the lower extremities of foals and alopecia (NRC, 1978). Zinc deficiency is known to cause dermatosis (Miller, 1989) and has been speculated to be a factor in the pathogenesis of NME and SND (see section 1.3.1).

1.6 SUMMARY

The black rhinoceros is a highly endangered species faltering in captivity. Efforts are underway to ascertain the exact causes for its decline in captivity. It is primarily a browser and may not adapt well to diets more suited to grazers like its cousin, the white rhinoceros.

Given the myriad of causes potentially responsible for NME and SND and the conflicting evidence supporting the various theories, the task before a researcher in identifying the pathology of these diseases in a new and relatively unstudied species (the black rhinoceros) is daunting. Based upon evidence in the literature, the essential fatty acids, n-6 linoleic acid and n-3 linolenic acid, were chosen as a reasonable starting place for the investigation into the nutritional problems of the captive North American black rhinoceros.

Both zinc and EFA deficiency cause membrane instability and altered prostaglandin metabolism and are implicated in NME/SND (Hansen, 1992). In light of the previous information, the diet of the captive black rhinoceros should be examined to determine the linoleic acid, n-3 linolenic acid, and n-6 linolenic acid content. Information gained from such work will either determine more accurately a cause of NME in the captive black rhinoceros or help to narrow the scope of the captive black rhinoceros' health problems through elimination.

CHAPTER II: ESSENTIAL FATTY ACIDS, TOTAL LIPID, AND TANNIN IN THE DIET OF THE CAPTIVE BLACK RHINOCEROS OF NORTH AMERICA AND IN BROWSES NATIVE TO ZIMBABWE, AFRICA

2.1 INTRODUCTION

The black rhinoceros is one of two African rhinoceros species that has been transported to United States zoos as a measure of protection from poaching. It is important that these animals thrive in captivity, for the future of the species as well as for the satisfaction of the millions of yearly zoo visitors whose money supports these animals. Unfortunately, as presented in Chapter I, the captive black rhinoceros in the United States has been afflicted with a skin disorder most closely resembling necrolytic migratory erythema (NME) in man and superficial necrolytic dermatitis (SND) in dogs.

Arguments suggesting that NME and SND are diseases of nutritional deficiencies were presented in Chapter I. Also presented in Chapter I were arguments suggesting that the current zoo diets fed to captive black rhinoceroses are more suited to grazers than to browsers. The existing literature does not conclusively demonstrate which of the nutritional factors might cause NME and SND, although there is evidence that grazers and browsers should be fed different diets.

The objective of the present study was to determine if diets fed to captive American black rhinoceroses had essential fatty acid (EFA) profiles similar to the EFA profiles of some of the browses preferred by wild black rhinoceroses in Zimbabwe, Africa. An estimation of the average captive American black rhinoceros' diet was determined by compiling total diet information supplied by 16 North American zoos. Twenty zoos supplied samples for EFA analysis. The rate of EFA loss was determined in several North American browses for the purpose of estimating overall EFA loss in the fresh African browses that are normally consumed by the wild black rhinoceros. A semi-quantitative estimate of tannin content was made of all samples received from rhino holding facilities and the African and North American browses collected for analysis using a colorimetric method.

2.2 MATERIALS AND METHODS

2.2.1 SAMPLE COLLECTION, TREATMENT, AND DIET CONTENT ANALYSIS

All North American zoos and black rhinoceros holding facilities belonging to the AZA and participating in the Species Survival Plan for the black rhinoceros were contacted through the director of the black rhinoceros SSP, Robert Reece, and asked to send approximately 1 lb. each of dry diet component (hays, pellets, supplements, etc.). Items prone to decay such as

produce and fresh browse were not requested. Participants also were asked to send a written description of the approximate amounts and types of all items given to their rhinoceroses on a daily basis, estimating the approximate feed intake of the rhinoceroses. Twenty zoos and/or black rhinoceros holding facilities responded over a 1 year period. The samples and items described were sorted into the following categories: Alfalfa Hay, Pellets, Grass Based Hay (included hays mixed with alfalfa), Produce, Fresh American Browse, and African Browse. The average captive black rhino's diet was estimated by computing the approximate percentage of listed items in each of the five categories in the diet descriptions provided, then averaging those percentages by category. Unidentified and unidentifiable samples were omitted from analysis.

Zimbabwean browse samples were collected by clipping 10-15 cm branches that were air-dried (to prevent fungal decay during shipping) and mailed to the United States in September of 1995. Table A.2 lists the species of browses collected from the Zambezi Valley and Harare areas of Zimbabwe, Africa. The samples were collected at the end of the dry season in 1995.

Fresh American browse samples for the essential fatty acid degradation study were collected in July of 1995 and extracted within 5 minutes of collection. The remaining portions of these samples were then air-dried and monitored for fungal infestation then stored for approximately 140 days. At this time they were ground to 2 mm mesh in conjunction with all other samples received and analyzed to determine loss of alpha linolenic acid and linoleic acid. All dried samples were stored in a dark cupboard at room temperature to prevent degradation of essential fatty acids by light exposure. All samples were ground to 2 mm mesh in a Wiley mill 1 week prior to beginning GC/MS analysis.This study was necessary to determine the losses of EFA concomitant with drying, storage, and exposure to oxygen and sunlight. All of these factors contributed to the loss of EFA in the dried African browse samples received for analysis. These losses had to be taken into account to allow for a realistic comparison of EFA intake between the diets.

The total contribution of lipid, EFA, and tannin by each category to the total North American captive black rhinoceros diet was calculated by averaging the measurement of interest over each category then multiplying that number by the percent that category contributed to the overall diet. These numbers were then added to give a total estimation of EFA, lipid or tannin content for the North American diet.

Three diets were proposed for comparison, a composite North American captive black rhinoceros diet, a wild black rhinoceros diet based on 100% consumption of fourteen African browses, and a speculative North American captive black rhinoceros diet based on 100% consumption of ten fresh North American browses. It should be noted that, although *Quercus rubra* (Red Oak) was included in the fresh North American browse analysis as a representative of oak spp., it should not be offered to black rhinoceroses as it has been linked to hemolytic anemia in horses (Duncan, 1961) nor should be *Acer rubrum* (Red Maple) (Tennant, et al., 1981).

Two sets of samples older than five years were generously donated for analysis by Ellen Dierenfeld, Ph.D. and Lee M. Bass. Unfortunately, due to the age of these samples and their storage in a ground condition, the EFA in them had degraded to the point of being unusable.

2.2.2 PERCENT DRY MATTER AND ETHER EXTRACTION

The percent dry matter (%DM) was determined in duplicate for each sample by drying a portion of each sample in a 100°C oven to a constant weight. This procedure is detailed in Appendix 1.1. Ether extraction was performed on all of the samples to determine the percent lipid in the samples using a Soxhlet extractor. This procedure is detailed in Appendix 1.2.

2.2.3 FATTY ACID EXTRACTION, METHYLATION, AND ANALYSIS

All samples were digested and methylated using a modified version of a micro-extraction method developed by Browse, et al. (1980). The digestion and methylation products from each sample were analyzed using a GC/MS (see Figure 2.1) to qualify and quantify the presence of linoleic acid, α-linolenic acid and γ-linolenic acid in the samples. These procedures are detailed in Appendices 1.3 and 1.4. Figures 2.2 -2.7 show chromatograms and mass spectra of standards and samples.

Figure 2.1 The Hewlett Packard GCD 1800A gas chromatograph/mass spectrometer used for all FAME analysis.

Figure 2.2 Chromatogram of prepared fatty acid methyl ester standard including the internal standard heptadecanoic acid methyl ester (22.15), and external standards linoleic acid methyl ester (22.92), gamma linolenic acid methyl ester (23.11), and alpha linolenic acid methyl ester (23.22). The FAME's are listed in order of their respective retention times (Rt's). Separation conditions are detailed in Appendix 1.4.

Figure 2.3 Chromatogram of a SAMPLE with heptadecanoic acid added as the internal standard. Separation conditions are detailed in Appendix 1.4. Rts as follows: ISTD = 22.19, C18:2n6 = 22.97, C18:3n3 = 23.29. C18:3n6 not found.

Figure 2.4 Mass spectra of internal standard, heptadecanoic acid methyl ester. Note *m/z* of the parent ion corresponds to the calculated molecular weight of 284 amu for heptadecanoic acid methyl ester. The mass spectra were used as a secondary means of identification. Separation conditions are detailed in Appendix 1.4.

Figure 2.5 Mass spectra of external standard linoleic acid methyl ester. Note *m/z* of the parent ion corresponds to the calculated molecular weight of 294 amu for linoleic acid methyl ester. Separation conditions are detailed in Appendix 1.4.

Figure 2.6 Mass spectra of external standard gamma linolenic acid methyl ester. Note *m/z* of the parent ion corresponds to the calculated molecular weight of 292 amu for gamma linolenic acid methyl ester. Separation conditions are detailed in Appendix 1.4.

Figure 2.7 Mass spectra of external standard alpha linolenic acid methyl ester. Note that the *m/z* of the parent ion corresponds to the calculated molecular weight of 292 amu for alpha linolenic acid methyl ester. Separation conditions are detailed in Appendix 1.4.

Figure 2.8 Mass spectra of two peaks eluting at the retention times corresponding to those of the linoleic acid methyl ester standard and the heptadecanoic acid methyl ester standard. Note that the *m/z* of the parent ions corresponds to the calculated molecular weight of the FAMEs of interest. Separation conditions are detailed in Appendix 1.4.

2.2.4 CONDENSED TANNIN APPROXIMATION

Condensed tannin content was approximated in each dried and ground sample after the digestion, methylation and extraction procedure was completed. The methanolic HCl phase in each vial in which methylation/digestion had just occurred was inspected for degree of red color intensity. This procedure was based on the fact that condensed tannins polymerize further upon heating with strong acids, producing red amorphous compounds known as phlobaphenes and small quantities of anthocyanidins (Griffiths, 1991). Each sample was judged for color intensity on a scale of 0-3, with 0 being no visible tannin coloration of the extract (no red color) and 3 being the most visible tannin coloration of the extract (darkest red color). Figure 2.9 depicts the color scale used. This method was not used for structural or chemical identification of the tannins. It was only used as a semi-quantitative approximation for a general comparison of condensed tannin content in the different samples. Hydrolyzable tannins were not taken into consideration.

Figure 2.9 Rating scale used for colorimetric assay of condensed tannin content. Samples were rated after digestion with methanolic HCl during the fatty acid methylation and extraction procedure.

2.2.5 CALCULATIONS

2.2.5.1 % Dry Matter (%DM)

$$\% \text{ DM} = \frac{(\text{Dried Thimble \& Sample Wt.}) - (\text{Empty Thimble Wt.})}{\text{Sample Wt. Before Drying}} * 100$$

2.2.5.2 Ether Extract Determination (%EE)

$$\%\text{EE} = \frac{(\text{Dried Thimble \& Samp. Wt.}) - (\text{Ext. Thimble \& Samp.Wt.})}{\text{Dry Sample Wt.}} * 100$$

2.2.5.3 Fatty Acid Quantification

peak area is unitless

amu = atomic mass units

Molecular Weight (MW) of heptadecanoic acid = 270 amu

MW of heptadecanoic acid methyl ester = 284 amu

MW of linoleic acid = 280 amu

MW of linoleic acid methyl ester = 294 amu

MW of linolenic acid = 278 amu

MW of linolenic acid methyl ester = 292 amu

Internal Standard (ISTD) = Heptadecanoic acid

Internal Standard methyl ester = ISTD-me

Stock ISTD concentration = 1.0 mg/mL

ISTD spike volume = 200 µL

Weight of ISTD per spike = (spike volume) * (ISTD concentration)

= 200 ~~µL~~ * (10^3~~mL~~/10^6~~µL~~) * 1.0mg/~~mL~~

= 0.20 mg

Weight of ISTD after methylation = (weight of ISTD per spike)*$\frac{(\text{MW ISTD-me})}{(\text{MW ISTD})}$

= 0.20 mg * (284 ~~amu~~/270 ~~amu~~) = 0.21 mg

Total sample volume = 1.0 mL

ISTD in sample concentration = weight of ISTD-me/total sample volume

= 0.21 mg/1.0 mL = 0.21 mg/mL

Concentration of EFA in each sample:

$$\text{mg/mL EFA} = \frac{(\text{amu EFA}) * (\text{peak area EFA}) * (\text{mg/mL ISTD})}{(\text{amu ISTD}) * (\text{peak area ISTD})}$$

FAME to EFA conversion:

mg/mL EFA = (mg/mL FAME) * (MW EFA/MW FAME)

For example in the quantification of linoleic acid given ISTD methyl ester peak area 1900207 and linoleic acid methyl ester peak area 4344091, wet sample weight 29.2 mg, % DM 91.5, and % EE 3.65:

mg/mL EFA-me = $\frac{294\ \text{~~amu~~}}{284\ \text{~~amu~~}} * \frac{4344091}{1900207}$ * 0.21 mg/mL ISDT-me

= 0.497 mg/mL linoleic acid -me

mg EFA-me = 0.497 mg/~~mL~~ * 1.0 ~~mL~~ total sample volume

= 0.497 mg linoleic acid -me

mg EFA = 0.497 mg EFA-me * 280 ~~amu~~/294 ~~amu~~

= 0.473 mg linoleic acid

µg EFA = 0.473 ~~mg~~ EFA * 10^6 µg EFA/10^3 ~~mg~~ EFA

= 473 µg linoleic acid

Dry sample weight = 0.0292 g * 91.5% = 0.0267 g

ppm EFA on a DM basis = (473 ~~µg~~/0.0267 ~~g~~) * (1.0 ~~g~~/10^6 ~~µg~~)

= 0.0177 ppm linoleic acid

Weight of lipid in sample = %EE * sample weight

= 3.65% * 0.0267 g

= 8.98×10^{-4} g of lipid in sample

% EFA of total lipids= EFA weight ÷ weight of lipid in sample

$= (473\ \mu g / 8.98 \times 10^{-4}\ g) * (1.0\ g / 10^{6}\ \mu g) * 100 = 53\%$

In this particular sample, linoleic acid accounted for 53 % of all lipids present.

2.2.5.4 Calculation of Daily EFA Intake by Captive Black Rhinos Consuming the Composite North American Diet

The gram intake of EFA on a dry matter basis for captive North American black rhinos was determined by estimating the average feed intake of the composite captive black rhino diet from the diet descriptions, determining what the dry matter intake was, then calculating how much each category's contribution of EFA was to the total diet. The following is an example of the determination of total EFA intake per rhino per day for the captive North American diet. Values used for the calculations in this section and sections 2.2.5.5 and 2.2.5.6 for %DM, %EE, and the percent of each EFA of total lipids can be found in Tables A.11, A.12, and A.13. Estimated total feed intake per day for captive black rhinos was determined by averaging the estimates provided by the black rhino holding facilities.

Estimated average total feed intake per day per rhino = 27.7 kg/day

Estimated average total dry matter intake per day per rhino = intake * %DM

= 27.7 kg * 81.4%

= 22.5 kg/day

Estimated average amount of linoleic acid in captive diet (from Table A.12)

= 16% of total lipid

Estimated average amount of linolenic acid in captive diet (from Table A.13)

= 13% of total lipid

Estimated average amount of lipid in captive diet = DM intake * %EE

= 22.5 kg * 2.1%

= 472 g

Total intake of linoleic acid per captive North American black rhino per day

= %linoleic acid of total lipids * 472 g of lipids ingested per day

= 76 g

Total intake of linolenic acid per captive North American black rhino per day

= %linolenic acid of total lipids * 472 g of lipids ingested per day

= 61 g

2.2.5.5 Calculation of Daily EFA Intake by Black Rhinos Consuming a Potential Fresh African Browse Diet

Wild adult black rhino intake after capture and residence in a boma is approximately 30 kg per day (Emslie and Adcock, 1994b); therefore, thirty kg was used in approximating EFA intake for African and North American browse based diets. This compared favorably with the estimate of 27.7 kg of feed intake in captive black rhinos in North America. The %DM for the African browses in their fresh state was estimated at 40% based on data compiled by Dierenfeld, et al. (1995) and Loutit, et al. (1987). Because these samples were received in a dry condition the loss of EFA between fresh and dried browse had to be accounted for in order to ensure that the total intake of EFA in wild African rhinoceroses was being accurately portrayed. Sections 2.2.1 and 2.3.1 detail the determination of degradation of EFA as performed in this study. Loss of EFA must be assumed in this case due to the condition of the samples and their exposure to sunlight, oxygen, and drying.

Approximate loss of linoleic acid = 40%

Approximate loss of linolenic acid = 90%

DM intake per day = 12 kg

Total lipid intake per day = 408 g

Total intake of linoleic acid per day based on dried African browse analysis:

= 8 g

Total intake of linoleic acid per day based on dried African browse analysis:

= 24 g

Taking into account 40% loss, total intake of linoleic acid per day of a wild black rhino consuming fresh browse would be:

X g = (8 g * 100)/60

= 13 g linoleic acid ingested per day

Taking into account 90% loss, total intake of linolenic acid per day of a wild black rhino consuming fresh browse would be:

X g = (24 g * 100)/10

= 240 g linolenic acid ingested per day

2.2.5.6 Calculation of Daily EFA Intake by the Black Rhinoceros Consuming a Speculative Fresh North American Browse Diet

Wild adult black rhino intake after capture and residence in a boma is approximately 30 kg per day (Emslie and Adcock, 1994b); therefore, thirty kg was used in approximating EFA intake for African and North American browse based diets.

DM intake per day = 30 kg * 29.8%DM = 9 kg

Total lipid intake per day = 9 kg * 3.1% = 270 g

Total intake of linoleic acid per day:

= 270 g * 5%

= 14 g/day

Total intake of linoleic acid per day:

= 270 g * 61%

= 165 g/day

2.3 RESULTS

2.3.1 DEGRADATION OF EFAS IN FRESH BROWSE

Linoleic acid and alpha linolenic acid both degraded after approximately 140 days of storage. The average overall loss of 40% of linoleic acid in the ten browses was not as much as the average overall loss of 90% of alpha linolenic acid. These overall losses were used to compute the amount of EFA in fresh African browse using figures obtained from dried African browse analysis. Figures 2.15 and 2.16 show the amounts of both fatty acids as percentages of total lipids at day 0 (fresh) and day 140 (dried). Tables A.16 and A.17 contain the original data.

2.3.2 DIET MAKEUP OF CAPTIVE BLACK RHINOCEROSES IN NORTH AMERICA AND WILD BLACK RHINOCEROSES

Table A.1 lists all zoos participating in this project and the extent of their participation. The average diet of the North American captive black rhinoceros consisted of approximately 40% alfalfa hay, 27% pelleted type feeds, 20% grass based hay, 8% produce, and 5% fresh browse. Figure 2.10 shows the breakdown of the captive North American black rhinoceros diet by category. The diet of wild black rhinos was assumed to consist entirely of fresh browse. Table A.2 lists all African browses analyzed. Table A.3 lists all North American browses analyzed. Tables A.4 through A.7 list all semi-dry type samples (hays, pellets, etc.) received from North American black rhinoceros holding facilities and the attendant original data. The information from the diet descriptions provided from each facility was translated into five categories (Alfalfa Hay, Grass Based Hay, Pellets, Produce, and Fresh North American Browse) and is

listed in Table A.10 as the percent found in the diet of each category from each facility. Also located in Table A.10 is the approximate daily feed intake per rhino from each facility. The category of Grass Based Hay included grass hays mixed with alfalfa. Tables A.19 and A.20 list all of the types of browse and produce fed to captive black rhinos from facilities participating in this study.

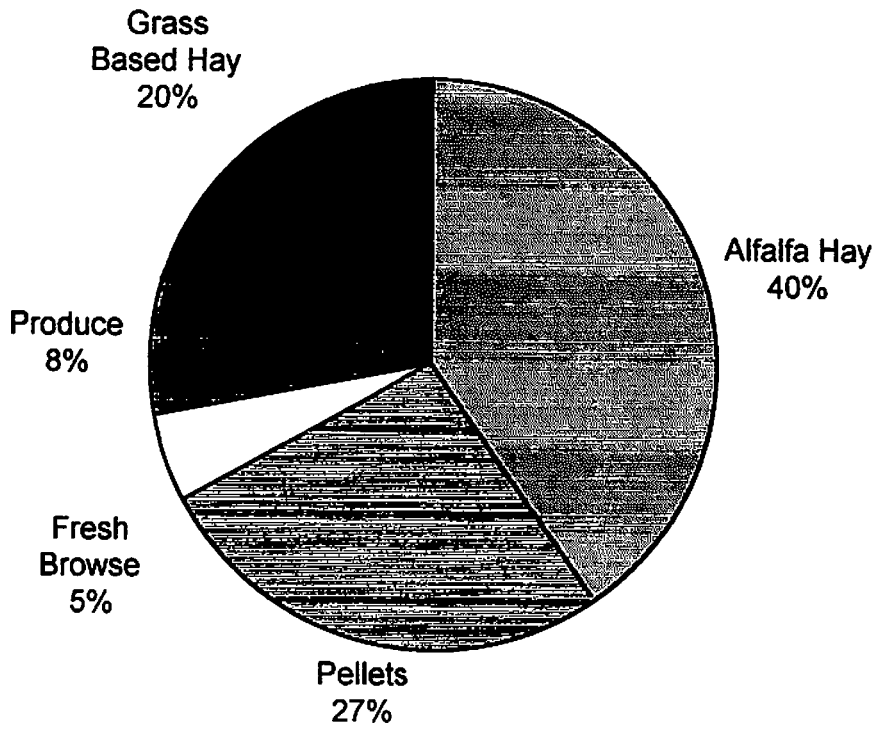

Figure 2.10 Estimation of the components of the composite North American captive black rhinoceros diet. Note the predominance of hay (forages, >60% of the total) of all types in the diet in contrast to 100% fresh browse in the wild rhino's diet.

2.3.3 ETHER EXTRACTION/TOTAL LIPID CONTENT

The estimated amount of lipid as a percent of total intake for captive black rhinos was 2.1% while the estimated daily amount of lipid intake in grams was 472 g. The postulated African browse diet was determined to be 3.4% lipid which translates to an intake of approximately 408 g of fat per day. The third diet, 100% fresh North American browse, consisted of 3.1% ether extract making the estimated total lipid intake per day of this diet approximately 270 g. Table A.11 contains the average % DM and average % EE by captive dietary category, for African browse, fresh North American browse, and the estimation of % DM and % EE for the composite North American captive black rhinoceros diet. Figures 2.11 depicts the differences in %EE among the three diets, while Figure 2.12 portrays the differences in total daily lipid intake of black rhinos consuming the three diets.

2.3.4 ESSENTIAL FATTY ACID ANALYSIS

Linoleic acid made up 16% of total lipids of the North American captive diet while alpha linolenic acid made up 13%. In dried African browse, linoleic acid was 2% and alpha linolenic acid 6% of total lipids. In fresh North American browse linoleic acid consisted of 5% of total lipids and alpha linolenic acid 61% of total lipids. Gamma-linolenic acid was not found in significant amounts in any of the samples. Using the estimates of total feed intake, total lipid intake per day, the daily intake amounts of linoleic acid and alpha-linolenic acid were calculated for black rhinos consuming the three postulated diets. The daily intakes of linoleic acid were respectively, 76 g, 13

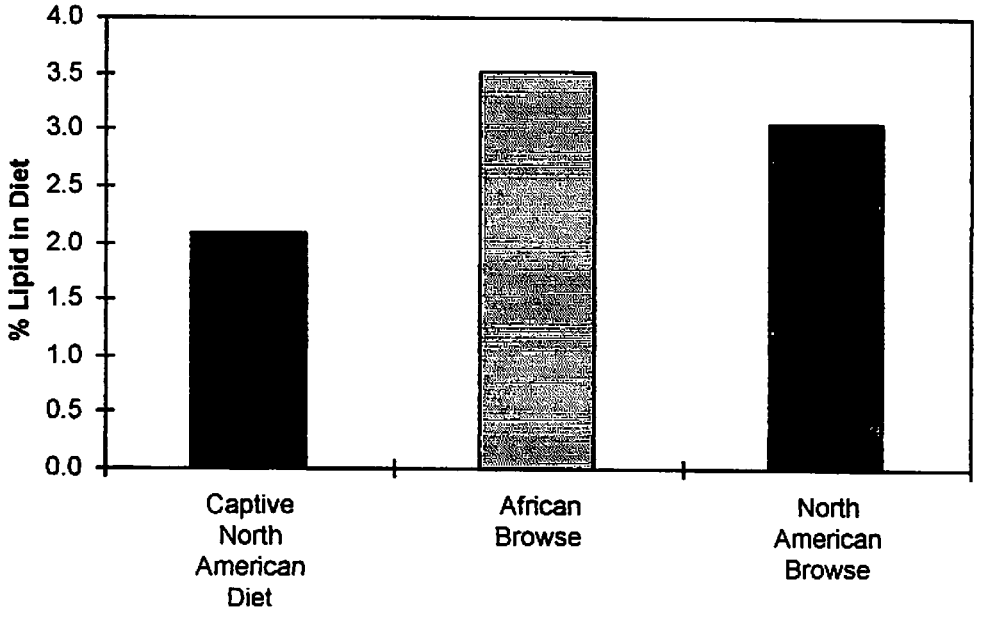

Figure 2.11 Total lipid content of three postulated black rhinoceros diets.

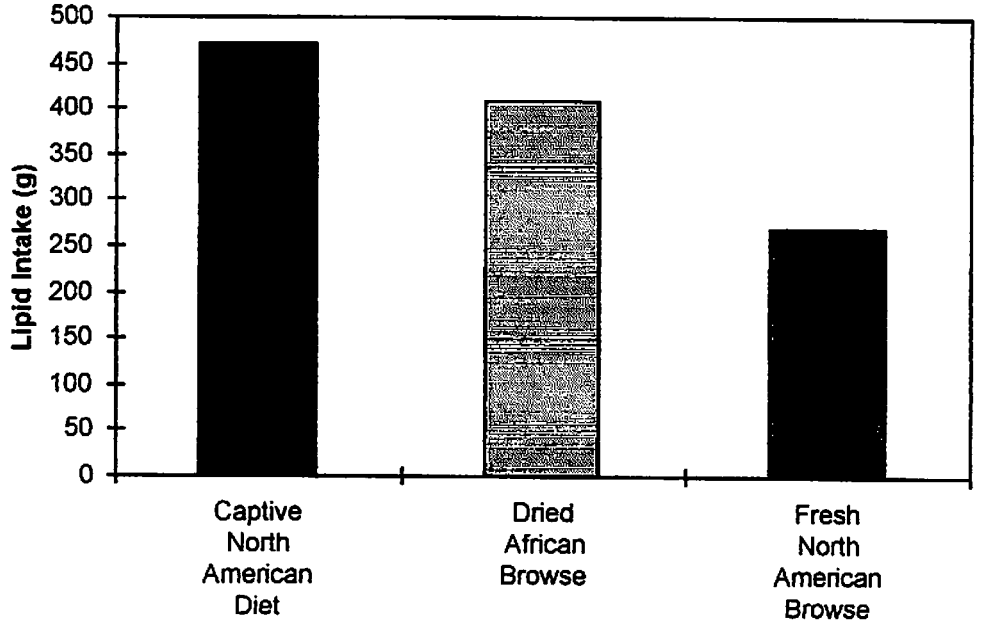

Figure 2.12 Total daily lipid intake in grams by black rhinos consuming three speculative diets.

g, and 14 g for the composite captive North American black rhinoceros diet, African browse corrected for EFA degradation, and fresh North American browse. The daily intakes of α-linolenic acid were respectively, 61 g, 240 g, and 165 g for the composite captive North American black rhinoceros diet, African browse corrected for EFA degradation, and fresh North American browse. These results are shown in Figures 2.13 and 2.14. The original data used to determine these values is located in Tables A.4 through A.7, A.9, A.11 through A.13, A.16, and A.17.

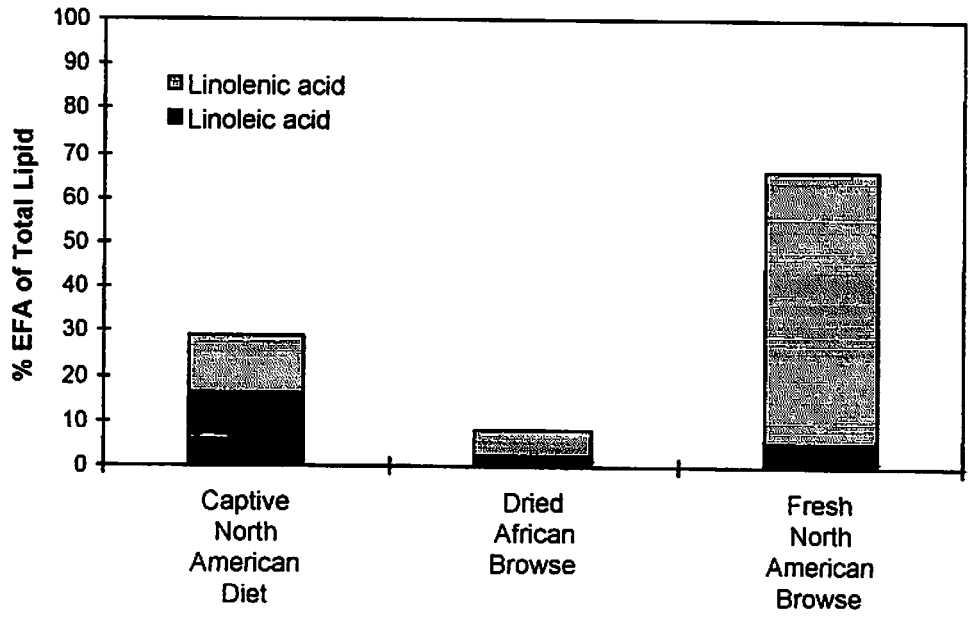

Figure 2.13 A comparison of linoleic acid and alpha linolenic acid as percentages of total lipids. Note the predominance of α linolenic acid in the fresh North American browse and the difference in the ratio of the two EFAs in the browse diets as opposed to the North American captive diet.

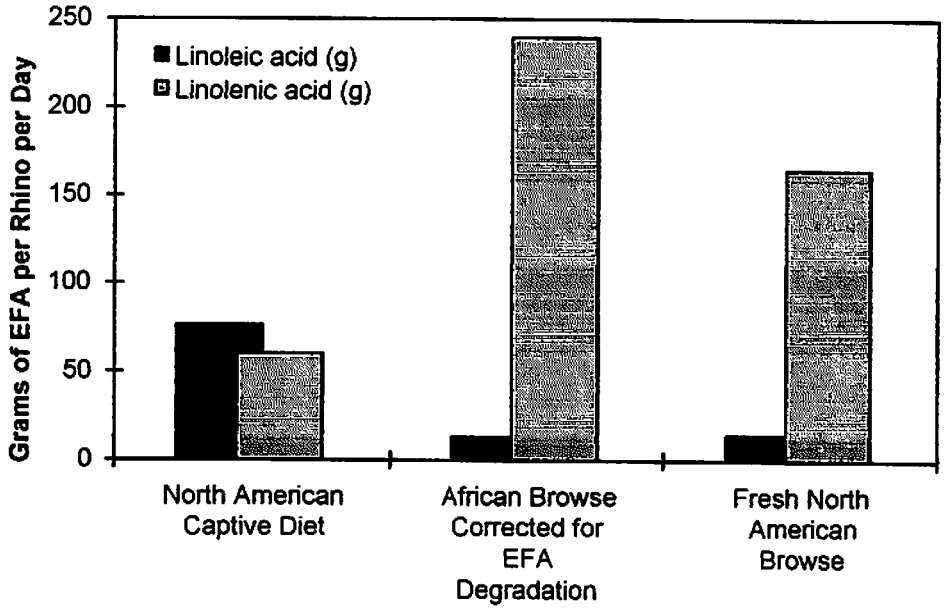

Figure 2.14 Intake of EFA per black rhinoceros per day in three possible diets. Note the predominance of α-linolenic acid in the fresh browse-based diets.

2.3.5 CONDENSED TANNIN APPROXIMATION

Condensed tannin content in ten North American browses averaged a relative value of 1.9 out of 3.0, while 2.1 out of 3.0 and 0.5 out of 3.0 were the values determined for the average of 14 African browses and the composite North American captive black rhinoceros diet. Figure 2.17 depicts the difference in condensed tannin content between the three diets. The condensed tannin content in the composite captive black rhinoceros diet in North America was minimal. Tables A.4 - A.7 and A.18 contain the data pertinent to the condensed tannin analysis.

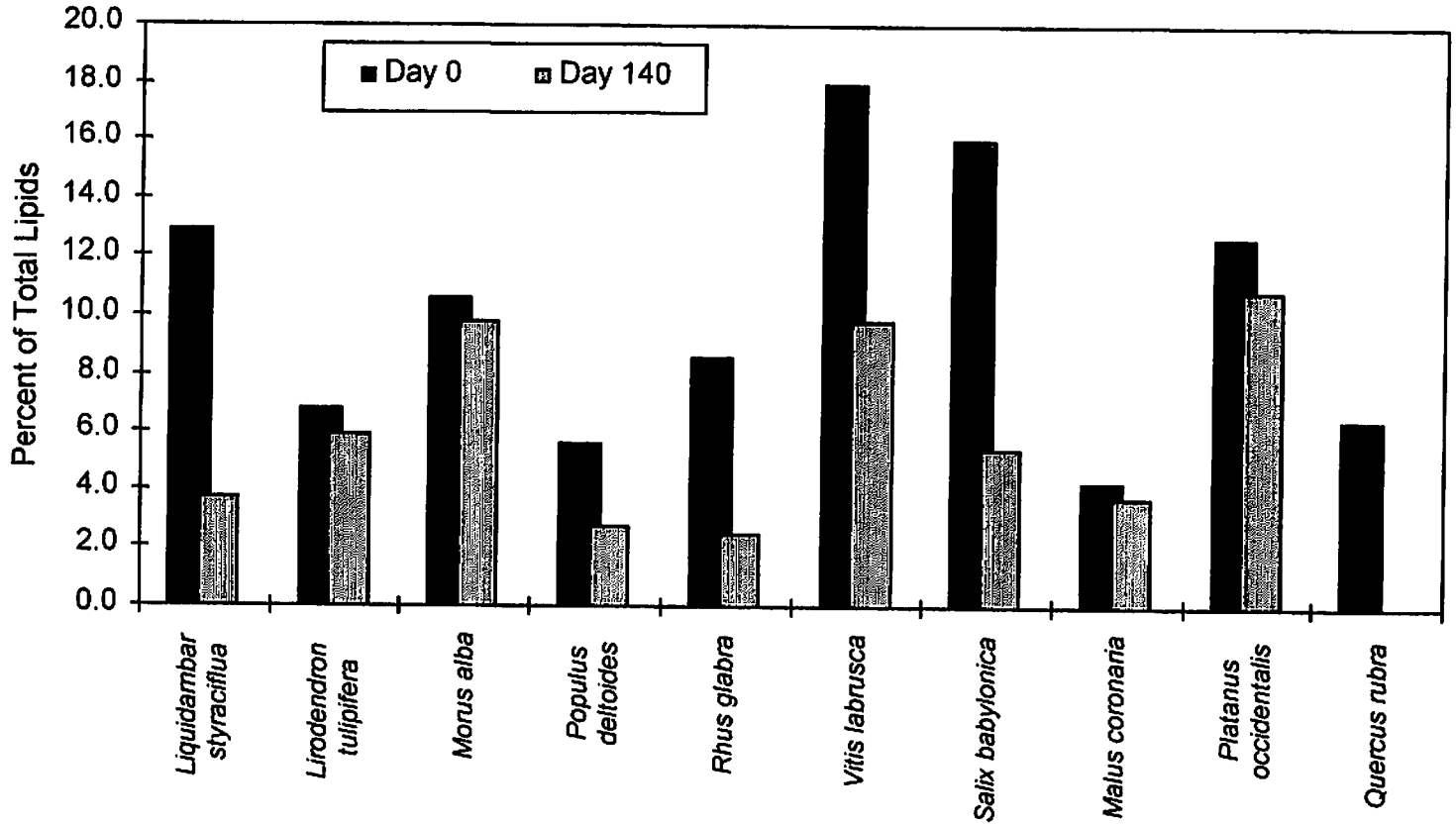

Figure 2.15 Degradation of linoleic acid in ten fresh North American browses. The columns indicate the amount of fatty acid as a percentage of total lipids. The fatty acid was undetectable in *Quercus rubra* after 140 days of storage.

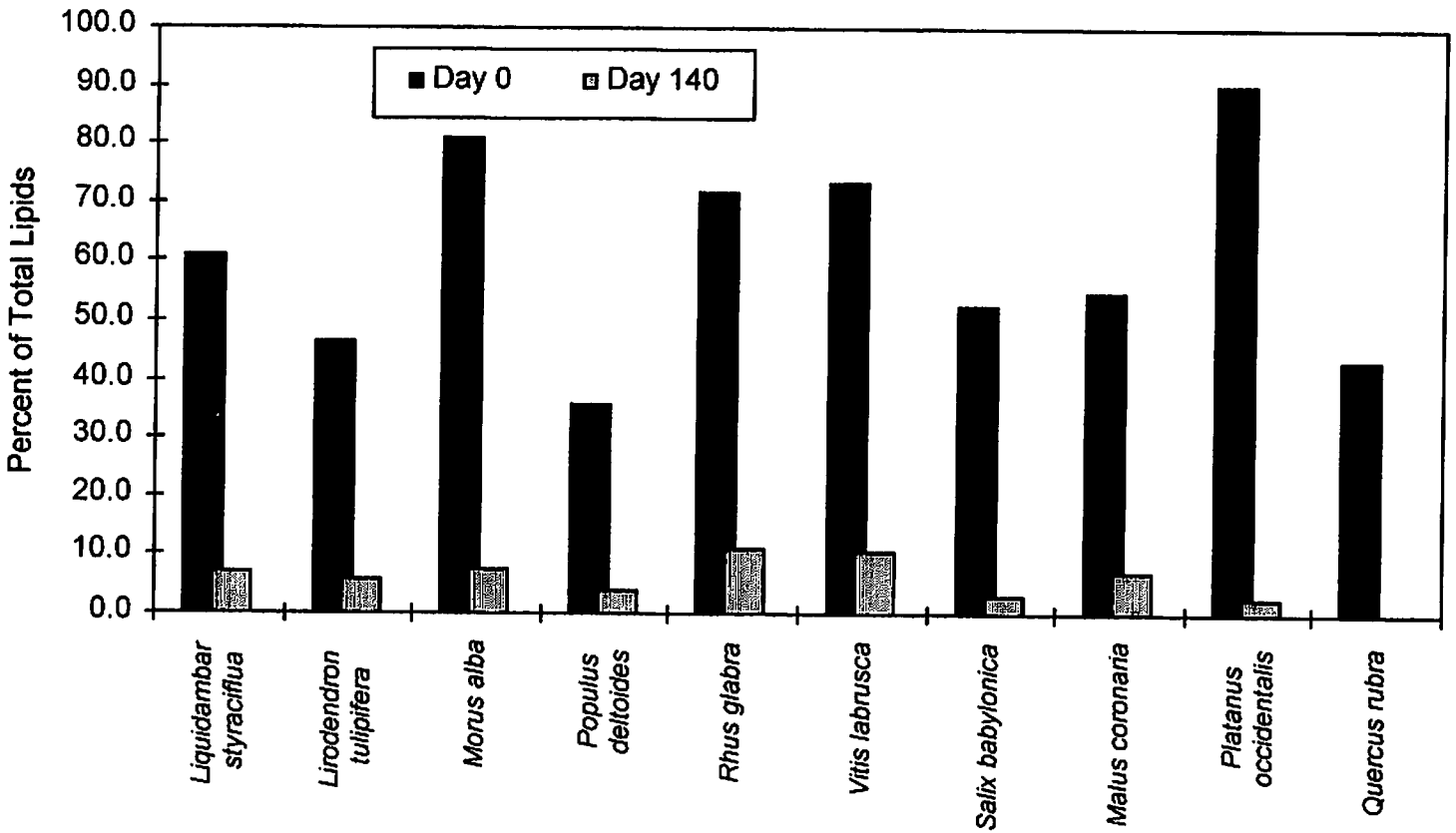

Figure 2.16 Degradation of alpha-linolenic acid in ten fresh North American browses. The columns indicate the amount of fatty acid as a percentage of total lipids. The fatty acid was undetectable in *Quercus rubra* after 140 days of storage.

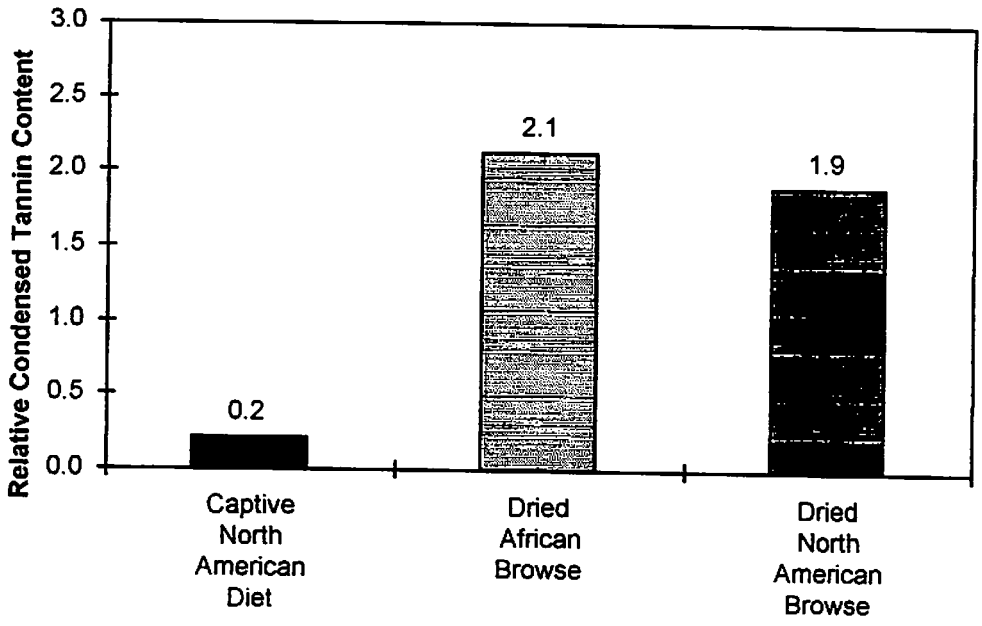

Figure 2.17 A comparison of condensed tannin content in three potential black rhinoceros diets. The diets were rating on a scale of 0-3 with 3 being the most condensed tannin. Note the high levels of condensed tannins in the browse based diets, approximately ten times that of the composite diet..

2.4 DISCUSSION

2.4.1 DIET

2.4.1.1 Diet Survey Information

The information received from the participating facilities was used to determine a composite diet of captive North American black rhinos. This composite diet was primarily used to determine the EFA and lipid intake of an average captive North American black rhino. The primary difference to remark upon in the comparison of the composite diet with the browse-based black rhino diets is the radical difference in the amount of browse consumed, 5% versus 100%. It has been postulated that forage based diets can be detrimental to animals which are primarily browsers (Ghebremeskel, et al., 1988). It appears inescapable that black rhinos must be fed hay of some type, but perhaps the inclusion of browse in captive North American black rhinoceros diets to a somewhat greater extent would be beneficial. When bringing in new black rhinoceroses, game ranches in Africa offer substantial amounts of various types of browse along with alfalfa hay and pellets (Emslie and Adcock, 1994b). Emslie and Adcock also recommend offering cut browse, sprayed with water to prevent wilting, at least twice a day (1994b). It is particularly important to avoid feeding wilted *Prunus* spp. due to the presence of cyanogenic glycosides (Cheeke and Shull, 1985) and *Taxus* spp. (yew, a common landscaping shrub) due to the presence of diterpenoid taxanes such as taxol (Cheeke, 1998). In fact, all browse species should be thoroughly investigated as to their chemical content and possible toxicity before being offered to black rhinos or any other herbivore.

This information can also be used for determining other nutritional levels of the captive black rhinoceros in North American. For example, most nutrient values for the three major components (alfalfa, pellts, grass based hay) are readily available. Data from African browse species could quickly be compared to information derived from this composite diet for a rapid check to determine the feasibility of a study in a particular area. It is also a good base to determine where changes and improvements in the diet can be made. For example, if a North American black rhinoceros holding facility notes that it has never seen a case of NME, but its ratio of alfalfa to grass based hay is 1:2 instead of 2:1 as seen in the average diet, it could report these findings to the black rhino community.

2.4.1.2 Total Lipid and EFA in Three Potential Black Rhinoceros Diets

Total lipid intake for the three diets (composite, African browse, and North American browse) was quite different (535g, 408g, 270g) because of the difference in DM intake per day. The composite diet has a much higher %DM than either of the fresh browse based diets. The differences between the diets becomes even more apparent after inspection of the intake of EFA. Linolenic acid intake is more than ten times that of linoleic acid in both the North American browse based diet and the African browse based diet after correction for EFA degradation. Contrarily, intake of linolenic acid from the composite diet was less than that of linoleic acid and less than the linolenic acid intake would be from either of the two browse diets. This would lead to some speculation that the captive black rhino in North America may not be meeting its α-linolenic acid requirements. Unfortunately, due to their complicated nature, these requirements have not been established in humans, much less black rhinos (Simopoulos, 1989). Assumption of an α-linolenic acid deficiency in the captive black rhinoceros in North America would not necessarily explain the symptoms currently experienced by them. Dermatitis cause by the deficiency of EFA can be rectified by supplementation with linoleic acid alone (Holman and Johnson, 1981).

An imbalance of linoleic acid and linolenic acid favoring linoleic acid has been found to be detrimental to human health (Fayard,1992), but the effects are not clear in other species. As it has been proven that diets imbalanced between linoleic acid and linolenic acid favoring linolenic acid cause harmful effects in humans, such as prolonged bleeding time (Willis, 1984), it stands to reason that the reverse imbalance is potentially harmful. Diets high in marine oils (which are high in n-3 fatty acids and metabolites of the omega three family) cause suppression of the immune system in mice and rabbits (FAO, 1994). It has also been demonstrated that diets with a high ratio of n-6 to n-3 fatty acids are damaging to the PUFA composition of developing human central nervous systems because high doses of linoleic acid have an inhibiting effect on n-3 fatty acids (Simopoulos, 1989). A diet high in linoleic acid would favor production of the 2-series prostaglandins and 4-series leukotrienes acid (Marshall and Johnston, 1981; Fayard, 1992, FAO, 1994). The consumption of a diet high in marine-developed n-3 PUFA (α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid) has been shown to decrease the production of pro-inflammatory cytokines and pro-aggregatory eicosanoids which contribute to the pathogenesis of inflammatory and atherosclerotic diseases (FAO, 1994). A study of tissue levels of cytokines and eicosanoids in healthy captive and NME afflicted black rhinos might reveal a connection between cytokines and eicosanoids in the promotion of NME. Another study spawned by this information might be to determine cytokine and eicosanoid tissue levels in captive black rhinos on a high linoleic acid/low α-linolenic acid diet with levels in wild black rhinos consuming low linoleic/high α-linolenic acid diets to determine if a high linoleic acid/low α-linolenic acid acid diet has an effect on

cytokine and eicosanoid levels that would cause the black rhinos to be prone to disease.

Finally, γ-linolenic acid was not found in significant amounts in the analysis of any of these samples. Based on these results, the black rhinoceros most likely does not have a requirement for γ-linolenic acid.

2.4.2 DEGRADATION OF EFA IN FRESH NORTH AMERICAN BROWSE

The results of this analysis were primarily used to determine the losses of EFA in the dried African browses received for EFA determination in order to have an closer approximation of EFA intake in wild black rhinos. A direct analysis of fresh browse would have been better for comparison, but this approximation was within the means of this study. Both EFA underwent substantial degradation during the process of drying and storage. This agrees with observations of EFA degradation by Ghebremeskel, et al. (1991). Linolenic acid underwent a more severe degradation than did linoleic acid which agrees with previous studies that have shown that (n-3) PUFA is highly oxidizable (FAO, 1994).

Lipid degradation is of concern in the diet of captive black rhinos because greater than 80% of their diet consists of material that has been exposed to conditions favoring oxidative damage to the cells of the plant material such as drying, wilting, heat extrusion (pellets) and exposure to oxygen. An animal eating fresh browse would potentially have a far smaller intake of degradation products such as free radicals. In addition, laboratory animals fed high levels of (n-3) PUFA have been shown to be prone to increased free radical activity, the risks of which can be minimized by increased intake of antioxidant nutrients such as Vitamin E (FAO, 1994). Free radicals can react with metal catalysts (especially with iron) and lipid hydroperoxides in a Fenton-type reaction to produce more reactive species (Miller and Brzezinska-Slebozinska, 1993). This is a potential problem as captive black rhinos have been noted to have tissue accumulation of iron (Miller, 1994) which, if combined with a high intake of free radicals or oxidatively damaged lipids would leave it prone to oxidative stress. Lipid peroxidation causes the formation of mutagenic lipid epoxides, lipid hydroperoxides, lipid alkoxyl and peroxyl radicals, and enals (Ames, et al., 1993) all of which are damaging compounds. Captive black rhinos in North America are also subject to progressive loss of vitamin E, an antioxidant. Vitamin E would be used extensively by animals consuming large amounts of free radicals and oxidatively damaged lipids, but can be regenerated by vitamin C (Miller and Brzezinska-Slebozinska, 1993). A third characteristic of black rhinos that might cause them to be inclined to oxidative stress is their catalase deficiency (Paglia, 1993, Paglia and Miller, 1993). Catalase is an enzyme which degrades hydrogen peroxide, the by-product of fatty acid degradation in peroxisomes and a very reactive molecule which causes oxidative damage to DNS when released into the cell (Ames, et al., 1993).

2.4.3 CONDENSED TANNIN CONTENT OF ITEMS IN THE DIETS OF WILD AND CAPTIVE BLACK RHINOCEROSES

Tannins may have an effect on the digestive physiology of the black rhinoceros which has not been predicted. Soluble tannins have been found to exist in all of the plant species chosen by wild black rhinos to a varying degree in one study (Loutit et al., 1987). Condensed tannins were found in appreciable levels in all African browses in this study while the levels found in the composite diet were minimal. Condensed tannins are not known to have a positive effect on nutrition of any species and it is not known if work has been done to determine if there is a detrimental effect caused by removing condensed tannins from the diet of an animal which normally consumes a highly tanniferous diet. Animals which regularly consume tanniferous diets are able to detoxify the tannins through the use of proline-rich salivary proteins and urea recycling (Van Soest, 1994).

2.4.4 CONCLUSION

The information received from the facilities participating in this study will be useful in evaluating the quality of nutrition currently being received by captive black rhinoceroses in North America. The composite captive black rhino diet in North America differed drastically from the browse based diet of wild black rhinos.

Linoleic acid intake in the composite diet was almost four times higher than in either browse based diet while α-linolenic acid was almost three times lower. This imbalance in the captive diet is highly significant and should be taken into consideration when considering the nutrition of the captive black rhinoceros in North America, especially given the possible effects this may have on the unexplored eicosanoid, prostaglandin, and leukotriene metabolism of the black rhinoceros. This data leads to speculation that α-linolenic acid requirements in the captive black rhinoceros in North America possily may not be currently met by the diet they are being offered. A deficiency of α-linolenic acid probably would not explain the symptoms of NME seen in black rhinos in North America. Gamma-linolenic acid was not found in significant amounts in any of the samples and probably is not linked to the current problems of captive black rhinos in North America.

EFA definitely undergoes degradation upon drying and storage. Degradation of lipids may exacerbate problems of oxidative stress in captive black rhinos in North America because of iron storage problems and progressive loss of vitamin E in captive black rhinos in North America, and catalase deficiency in the species as a whole.

Wild black rhinos consume many browses containing both soluble and condensed tannins. Beneficial effects of tannins is an area of research in which not much knowledge has accumulated.

This work has identified three potential areas of further research in the nutrition of captive black rhinos in North America and has clarified the differences between EFA intake in the composite North American diet and two

browse based diets, one of African browse and the other of North American browse. A study of tissue levels of cytokines and eicosanoids in healthy captive and NME afflicted black rhinos might reveal a connection between cytokines and eicosanoids in the promotion of NME. Another study spawned by this information might be to determine cytokine and eicosanoid tissue levels in captive black rhinos on a high linoleic acid/low α-linolenic acid diet with levels in wild black rhinos consuming low linoleic/high α-linolenic acid diets to determine if a high linoleic acid/low α-linolenic acid acid diet has an effect on cytokine and eicosanoid levels that would cause the black rhinos to be prone to disease. A study of the effects of condensed tannins on the nutrition of the black rhinoceros may also be appropriate, given the findings of this study. The two browse based diets were more similar in composition to each other than to the composite diet.

APPENDIX 1: METHODS

1.1 PERCENT DRY MATTER DETERMINATION

Materials Required:

Alundum Thimbles

Procedure

1. Place thimbles in 100°C oven overnight to remove moisture.
2. Remove thimbles from oven and place in a dessiccator until they reach room temperature. Weigh thimbles.
3. Weigh 2.0 - 3.0 g of ground sample (2 mm mesh) into alundum thimble.
4. Place samples in 100°C oven overnight or until a constant weight is achieved to remove all moisture.
5. Weigh sample and thimble after drying.

1.2 ETHER EXTRACT DETERMINATION

Chemicals Required

Ethyl Ether (FisherChemical, Pittsburgh, PA)

Materials Required:

Alundum Thimbles

Dry samples

1. Place thimbles containing dry samples from the procedure in 1.1 in Soxhlet extractor.
2. Turn on condenser water and heat source.
3. Adjust heat so ethyl ether fills soxhlet and drains about once every two hours (approximately 50°C). Add ether when necessary.
4. After three days, turn off heat as soon as soxhlet drains, remove thimbles, and place them under the hood until ether completely evaporates.
5. Dry samples in 100C oven overnight. Weigh back.

1.3 FATTY ACID TISSUE EXTRACTION AND ESTERIFICATION

This procedure is a modification of the procedure developed by Browse, et al. (1986). The procedure involves the simultaneous digestion of lipid and methylation of the fatty acids into esters in each sample. The 2,2-dimethoxypropane is added to react with any water present and the BHT is added as an antioxidant to prevent degradation of the FAMEs. The samples are heated at 80°C for an hour to ensure complete digestion and methylation. The samples are centrifuged to break any emulsion formed and completely separate the phases. The fatty acid methyl esters (FAMEs) are then extracted into an organic phase of hexane from which they can be taken directly for GC/MS analysis. The internal standard, heptadecanoic acid was appropriate given that the analysis was of plant materials that do not commonly manufacture heptadecanoic acid.

Chemicals required:

Butylated Hydroxy Toluene (Sigma Chemical Co., St. Louis, MO)

2,2-Dimethoxypropane (Sigma Chemical Co., St. Louis, MO)

Heptadecanoic acid, 99% (Sigma Chemical Co., St. Louis, MO)

n-Hexane, 99+% (Sigma Chemical Co., St. Louis, MO)

Methanol, 99+% (Sigma Chemical Co., St. Louis, MO)

3 N Methanolic Hydrochloric Acid (Supelco, Inc., Bellefonte, PA)

Sodium Chloride (FisherChemical, Pittsburgh, PA)

Reagents Required:

0.9% Aqueous Sodium Chloride Solution

1 mg/mL Heptadecanoic acid in methanol

n-Hexane, 99+% (Sigma Chemical Co., St. Louis, MO)

1 N Methanolic Hydrochloric Acid with 5% 2,2-Dimethoxypropane and 50 µg/mL of Butylated Hydroxy Toluene

* Use distilled water to make all aqueous stocks.

Internal Standard Required

1 mg/mL Heptadecanoic acid in methanol

Materials Required:

Pipettor (200 µL and 1 mL)

Pipettor Tips

5 mL Reacti-vials with Teflon-lined Caps (Wheaton)

Small Spatula

Procedure

1. Weigh approximately 30 mg of ground sample (2 mm mesh) into a 5 mL reacti-vial.
2. Pipet 200 µL of the internal standard heptadecanoic acid onto the sample, followed by 1 mL of 1 N methanolic HCl solution, then purge with nitrogen, and seal.
3. Heat at 80°C for 1 hour to ensure the complete digestion of lipid and methylation of the fatty acids.
4. Remove samples from heat. When they have reached room temperature, pipet 1 mL of hexane and 1 mL of 0.9% NaCl solution.

5. Shake reacti-vials by hand for 30 seconds each to extract the fatty acid methyl esters into the hexane.
6. Centrifuge samples at 1000 *g* for 1 minute.
7. Take a 4 µL sample directly from the upper hexane phase for GC/MS analysis.
8. Store extracted samples in a freezer.

1.4 GC/MS ANALYSIS

Chemicals Required

Ultra High Purity Helium Gas (Empire Airgas, Inc., Elmira, NY)

n-Hexane, 99+% (Sigma Chemical Co., St. Louis, MO)

Heptadecanoic acid methyl ester, 95% (Sigma Chemical Co., St. Louis, MO)

Linoleic acid methyl ester, 99% (Sigma Chemical Co., St. Louis, MO)

Linolenic acid methyl ester, 99% (Sigma Chemical Co., St. Louis, MO)

Methanol, 99+% (Sigma Chemical Co., St. Louis, MO)

Materials Required

Hewlett-Packard Gas Chromatograph with a Mass Spectrometric Detector (GC/MS) HP GCD 1800A

30m x 0.32 mm ID fused silica capillary column with a 0.20 µm biscyanopropyl polysiloxane film (Supelco, Inc., Bellefonte, PA)

10 µL SGE International syringe (Supelco, Inc., Bellefonte, PA)

Standards Required

Heptadecanoic acid methyl ester in n-hexane

Linoleic acid methyl ester in n-hexane

Linolenic acid methyl ester in n-hexane

1. Take a 4 µL sample directly from the hexane phase of the extracted samples from the procedure in 1.3.

2. Inject the sample onto the GC column using the following temperature program:

Initial temperature:	50°C with a 5 min. hold
Rate:	20°C/min.
Final temperature:	200°C with a 7.5 min. hold

3. The split ratio was 87.5:1 and the carrier gas (helium) flow rate was 1 mL/min.
4. External standards of n-3 linolenic acid and n-6 linolenic acid methyl ester were used to differentiate between the two isomers.
5. External standards of all four fatty acids (17:0, 18:2n-6, 18:3:n-3, 18:3n-6) were used to build a spectral library for secondary identification by the mass spectrometric detector.
6. Heptadecanoic acid was used as an internal standard for quantification of all FAMEs.

APPENDIX 2: DATA TABLES

Table A.1 Zoos and Black Rhinoceros Holding Facilities Participating in Study. An "X" indicates that samples and/or a detailed description of the black rhino's diet were received.

Facility	Samples	Diet Description
Brookfield Zoo Chicago Zoological Society Brookfield, IL 60513	X	X
Busch Gardens Tampa FL 33674	X	X
Cincinnati Zoo 3400 Vine St. Cincinnati, OH 45220-1399	X	X
Dallas Zoo 621 East Clarendon Dr. Dallas, TX 75203	X	X
Denver Zoological Foundation City Park Denver, CO 80205-4899	X	X
Detroit Zoological Park Royal Oak, MI 48068-0039	X	
El Coyote Ranch Lee M. Bass 201 Main St. Fort Worth, TX 76102-3131	X	
Fossil Rim Wildlife Center Glen Rose, TX 76043	X	X

Table A.1 cont.

Facility	Samples	Diet Description
Lee Richardson Zoo 312 E. Finnup Dr. Garden City, KS 67846-0499	X	X
Los Angeles Zoo 5333 Zoo Drive Los Angeles, CA 90027	X	X
Metro Washington Park Zoo 4001 SW Canyon Rd. Portland, OR 97221-2799	X	
Miami Metrozoo 12400 SW 152nd St. Miami, FL 33177	X	X
Milwaukee County Zoo 10001 West Bluemound Road Milwaukee, WI 53226	X	X
Oklahoma City Zoological Park 2101 NE 50th Oklahoma City, OK 73111	X	X
Riverbanks Zoological Park and Botanical Gardens Columbia, SC 29202-1060	X	X
San Antonio Zoological Society 3903 N. St. Mary San Antonio, TX 78212	X	
Sedgewick County Zoo African Veldt 5555 Zoo Blvd. Wichita, KS 67212	X	X

Table A.1 cont.

Facility	Samples	Diet Description
White Oaks Conservation Center 726 Owens Rd. Yulee, FL 32097	X	X
The Wildlife Conservation Society 185th St. and Southern Blvd. Bronx, NY 10460	X	X
Zoo Atlanta 800 cherokee Ave. SE Atlanta, GA 30315	X	X

TABLE A.2 A list of the fourteen analyzed African browses and their common names where known. Keith Coates Palgrave's tome of south African trees was used as a reference to determine the common names of all species listed.

Scientific Name	Common Name
Acacia karroo	Sweet Thorn
Cassia abreviata	Long-tail Cassia
Combretum zeyheri	Large-fruited Bushwillow
Commiphora mossambicensis	Pepper-leaved Commiphora
Dalbergia melanoxylon	Hairy Flat-bean
Dichrostachys cineria	Sickle Bush
Diospyros quiloensis	Crocodile-bark Diospyros
Elephantorrhiza goetzii	Large-bean Elephant-root
Grewia monticola	Grey Grewia, Silver Raisin
Pterocarpus rotundifolius	Round-leaved Bloodwood
Schrebera trichoclada	Wooden-pear
Securanegra virosa	Snowberry Tree
Vitex petersiana	Not listed
Ziziphus mucronata	Buffalo-thorn

Table A.3 Scientific and common names of the ten analyzed North American browses. The Audobon Society's Eastern Forests guide (Sutton, 1993) was used as a reference to determine the common names of all species listed.

Scientific Name	Common Name
Liquidambar styraciflua	Sweetgum
Lirodendron tulipifera	Tuliptree, Yellow Poplar
Morus alba	White Mulberry
Populus deltoides	Cottonwood
Rhus glabra	Smooth Sumac
Vitis labrusca	Wild American Grapevine
Salix babylonica	Weeping Willow
Malus coronaria	Crabapple
Platanus occidentalis	Sycamore
Quercus rubra	Red Oak

Table A.4 Original data, including wet sample weight in grams, % dry matter, % ether extract, ISTD heptadecanoic Acid (17:0) peak area, linoleic acid (18:2n6) peak area, linolenic acid (18:3n3) peak area, and gamma linolenic acid (18:3n6) peak area, and colorimetric condensed tannin rating for the category of Alfalfa Hay. An (*) indicates that the sample was analyzed only once in the particular category due to a lack of available material.

Type of Sample	%DM	%EE	Tannin Rating	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Volume of ISTD Spike (µL)	Wet Sample Weight (g)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
Alfalfa Hay	91.9	2.76	0	2201046	443275	423000	200	0.0337	1343	1273
Alfalfa Hay	92.3	2.18	0	536923	127987	150034	200	0.0370	1448	1686
Alfalfa Hay	*84.8	*2.41	0	1828893	410977	440290	200	0.0359	1528	1626
Alfalfa Hay	*84.8	*2.41	0	541891	164442	187841	200	0.0437	1695	1924
Alfalfa Hay	*92.1	*2.52	0	1980188	335367	330861	200	0.0345	1104	1081
Alfalfa Hay	*92.1	*2.52	0	687466	105091	126835	200	0.0347	990	1187
Alfalfa Hay	91.1	3.05	0	1324913	383453	835430	200	0.0409	1602	3467
Alfalfa Hay	91.8	2.65	0	652017	153082	354690	200	0.0426	1248	2871
Alfalfa Hay	90.8	2.99	0	1676543	372534	548697	200	0.0372	1359	1988
Alfalfa Hay	91.2	1.97	0	632217	134155	204000	200	0.0437	1105	1669
Alfalfa Hay	91.1	3.04	0	1439152	408405	1077919	200	0.0355	1813	4752
Alfalfa Hay	91.5	2.40	0	587818	177367	509960	200	0.0397	1724	4922
Alfalfa Hay	91.5	2.28	0	1660695	272798	272929	200	0.0371	1001	994
Alfalfa Hay	91.7	1.35	0	668513	106925	93442	200	0.0413	875	760
Alfalfa Hay	91.0	2.15	0	2201389	423625	632585	200	0.0348	1256	1863
Alfalfa Hay	91.3	0.83	0	716886	148351	173623	200	0.0399	1178	1369
Alfalfa Hay	91.1	2.59	0	901433	200802	325709	200	0.0368	1367	2202
Alfalfa Hay	92.3	1.64	0	761891	183542	415594	200	0.0352	1545	3475

Table A.4 continued.

Type of Sample	%DM	%EE	Tannin Rating	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Volume of ISTD Spike (µL)	Wet Sample Weight (g)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
Alfalfa Cubes	91.1	2.97	0	896655	236964	337913	200	0.0378	1564	2215
Alfalfa Cubes	92.7	1.42	0	696358	161246	225885	200	0.0397	1305	1815
Alfalfa Cubes	93.9	1.88								
Alfalfa Hay	90.4	3.59	0	696977	159940	276598	200	0.0393	1309	2249
Alfalfa Hay	92.4	0.84	0	673104	116967	208734	200	0.0353	1104	1956
Alfalfa Hay	94.2	1.48								
Alfalfa Hay	92.8	2.58	0	733700	133937	371353	200	0.0369	1095	3017
Alfalfa Hay	94.2	2.58	0	777122	169398	346699	200	0.0372	1298	2638
Alfalfa Hay	90.2	1.50	0	824570	104542	127988	200	0.0335	851	1035
Alfalfa Hay	92.0	0.75	0	665618	116549	130141	200	0.0409	963	1068
Alfalfa Hay	94.0	1.26								
Alfalfa Hay	91.2	2.68	0	654784	101177	195723	200	0.0332	1035	1989
Alfalfa Hay	93.3	0.89	0	680625	142843	270282	200	0.0341	1369	2573
Alfalfa Hay	94.7	1.85								
Alfalfa Hay	89.5	0.70	0	603887	145638	279566	200	0.0359	1536	2928
Alfalfa Hay	91.6	1.08	0	629574	142481	296144	200	0.0381	1358	2804
Alfalfa Hay	91.0	0.77	0	625849	119551	185328	200	0.0391	1089	1677
Alfalfa Hay	92.7	0.86	0	633263	87859	135121	200	0.0369	838	1280
Alfalfa Hay	94.9	2.16								
Alfalfa Hay	88.5	0.98	0	613957	108902	247132	200	0.0358	1141	2572
Alfalfa Hay	91.3	1.26	0	696876	153621	281630	200	0.0394	1289	2346
Alfalfa Hay	91.0	2.11	0	627705	94982	0	200	0.0319	1059	0
Alfalfa Hay	94.5	1.69	0	610372	146788	381045	200	0.0409	1313	3384

Table A.5 Original data, including wet sample weight in grams, % dry matter, % ether extract, ISTD heptadecanoic Acid (17:0) peak area, linoleic acid (18:2n6) peak area, linolenic acid (18:3n3) peak area, and gamma linolenic acid (18:3n6)

peak area, and colorimetric condensed tannin rating for the category of Grass Based Hay. An (*) indicates that the sample was analyzed only once in the particular category due to a lack of available material.

Type of Sample	%DM	%EE	Tannin Rating	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Wet Sample Weight (g)	Volume of ISTD Spike (µL)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
Mixed Timothy and Alfalfa Hay	*93.2	*2.49	1	2383919	675658	1655455	0.0350	200	1799	4378
Mixed Timothy and Alfalfa Hay	*93.2	*2.49	1	653089	160683	496150	0.0318	200	1719	5271
Red Topped Cane	89.5	4.63	1	2223734	284156	0	0.0354	200	829	0
Red Topped Cane	90.8	2.44	1	615514	87655	0	0.0461	200	709	0
Bermuda Grass	*93.0	*0.88	0	1558639	203858	301600	0.0349	200	834	1226
Bermuda Grass	*93.0	*0.88	0	746900	104569	125157	0.0412	200	757	899
Mixed Species Prairie Grass Hay	92.6	2.22	2	1737617	184075	296685	0.0363	200	652	1043
Mixed Species Prairie Grass Hay	92.8	1.64	2	600605	68977	0	0.0431	200	595	0
Timothy Hay	93.0	2.69	0	2042902	315548	998436	0.0385	200	891	2799
Timothy Hay	93.5	1.19	0	582086	70752	231075	0.0388	200	696	2256
Coastal Bermuda Grass Hay	91.5	1.73	0	2342146	597125	1373885	0.0415	200	1384	3163
Coastal Bermuda Grass Hay	92.3	0.97	0	642476	129730	299210	0.0381	200	1194	2735

Table A.5 continued.

Type of Sample	%DM	%EE	Tannin	ISTDme	18:2n6me	18:3n3me	Wet	Volume of	ppm	ppm

			Rating	Peak Area	Peak Area	Peak Area	Sample Weight (g)	ISTD Spike (µL)	18:2n6 (µg/g)	18:3n3 (µg/g)
Mixed Timothy and Alfalfa Hay	91.4	3.11	0	2009982	443017	808568	0.0397	200	1252	2270
Mixed Timothy and Alfalfa Hay	92.2	1.50	0	691814	134993	244833	0.0376	200	1170	2108
2:1 Alfalfa:Mixed Species Prairie Grass Hay (Flowtron)	92.5	2.02	1	808638	134896	157373	0.0360	200	1032	1196
2:1 Alfalfa:Mixed Species Prairie Grass Hay (Flowtron)	93.4	1.23	1	791789	140936	288227	0.0381	200	1041	2114
Timothy Hay	91.7	3.54	0	726710	122817	284672	0.0343	200	1092	2514
Timothy Hay	93.7	1.30	0	675180	104184	219419	0.0407	200	840	1757
Timothy Hay	94.9	1.51								
Mixed Species Brome Grass Hay	91.6	0.86	0	662830	60591	81546	0.0373	200	545	728
Mixed Species Brome Grass Hay	93.0	0.94	0	758019	125166	100916	0.0413	200	889	712
Mixed Species Brome Grass Hay	94.8	1.38								
Mixed Species Prairie Grass Hay	90.6	1.04	0	679762	175189	455411	0.0413	200	1402	3619
Mixed Species Prairie Grass Hay	91.9	1.31	0	768348	188885	525538	0.0380	200	1453	4016

Table A.5 continued.

Type of Sample	%DM	%EE	Tannin	ISTDme	18:2n6me	18:3n3me	Wet	Volume of	ppm	ppm

			Rating	Peak Area	Peak Area	Peak Area	Sample Weight (g)	ISTD Spike (µL)	18:2n6 (µg/g)	18:3n3 (µg/g)
Mixed Species Prairie Grass Hay	94.0	1.59								
Mixed Species Prairie Grass Hay	92.6	0.95	1	650567	147882	412851	0.0394	200	1270	3522
Mixed Species Prairie Grass Hay	94.1	1.21	1	775054	164084	348032	0.0378	200	1233	2598
Mixed Species Prairie Grass Hay	95.4	2.00								
Timothy Hay	91.9	1.94	0	645838	90474	149508	0.0379	200	822	1349
Timothy Hay	94.3	1.57	0	494166	60825	141085	0.0428	200	640	1473

Table A.6 Original data, including wet sample weight in grams, % dry matter, % ether extract, ISTD heptadecanoic Acid (17:0) peak area, linoleic acid (18:2n6) peak area, linolenic acid (18:3n3) peak area, and gamma linolenic acid (18:3n6) peak area, and colorimetric condensed tannin rating for the category of Pellets. An (*) indicates that the sample was analyzed only once in the particular category due to a lack of available material.

Type of Sample	%DM	%EE	Tannin Rating	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Wet Sample Weight (g)	Volume of ISTD Spike (µL)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
ADF 25	*92.8	*2.78	0	2377728	3924932	732717	0.0326	200	11297	2095
ADF 25	*92.8	*2.78	0	595457	1163256	268106	0.0341	200	12781	2926
Elephant Supplement 9072 ACCO Feeds	91.8	3.16	0	2141904	4034885	0	0.0355	200	11942	0
Elephant Supplement 9072 ACCO Feeds	92.2	2.90	0	565367	929682	70680	0.0332	200	11146	842
Herbivorous Zoo	91.1	3.00	0	1825691	2707396	366286	0.0290	200	11583	1556
Herbivorous Zoo	91.7	2.00	0	638407	1327344	232882	0.0344	200	13691	2386
HMS Low Fiber	91.0	3.85	0	1900207	4344091	541728	0.0292	200	17715	2194
HMS Low Fiber	92.0	2.88	0	482141	1414113	242879	0.0331	200	20050	3420
Mazuri ADF 16	90.5	2.76	0	2106568	4163534	713302	0.0319	200	14128	2404
Mazuri ADF 16	91.1	2.27	0	635697	1532443	339907	0.0342	200	16072	3541
Mazuri ADF 16	90.5	3.28	0	1805342	2722297	593212	0.0289	200	11884	2572
Mazuri ADF 16	91.3	3.18	0	627252	1469102	391586	0.0382	200	13965	3697
Moose Pellet	91.2	2.69	0	1624417	2022680	152192	0.0334	200	8431	630
Moose Pellet	91.9	1.68	0	567498	1019537	93037	0.0426	200	9537	864
Nutrena ADF 16 Herbivore	89.6	2.31	0	1695466	3101869	483780	0.0319	200	13245	2052

Table A.6 continued.

Type of Sample	%DM	%EE	Tannin Rating	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Wet Sample Weight (g)	Volume of ISTD Spike (µL)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
Nutrena ADF 16 Herbivore	89.7	1.89	0	556040	809078	148949	0.0260	200	12925	2363
Mazuri ADF 16	90.4	2.76	0	1913905	3742258	698484	0.0355	200	12573	2331
Mazuri ADF 16	91.0	2.56	0	627385	908087	168839	0.0253	200	13059	2412
Mazuri ADF 16	90.9	3.09	0	1861855	3185233	675850	0.0321	200	12126	2555
Mazuri ADF 16	91.1	2.58	0	574748	891544	218168	0.0293	200	12045	2928
Mazuri ADF 16	90.4	2.82	0	2171780	5450787	962836	0.0405	200	14154	2483
Mazuri ADF 16	90.9	2.78	0	619505	1100253	175138	0.0239	200	16972	2683
Mazuri ADF 25	92.1	3.99	0	1954816	2726169	889014	0.0360	200	8713	2822
Mazuri ADF 25	92.0	3.45	0	628198	791123	229579	0.0226	200	12534	3612
Mazuri Elephant	91.5	2.97	0	447849	1112138	204628	0.0341	200	16460	3008
Mazuri Elephant	91.7	3.75	0	624235	1559775	365834	0.0341	200	16562	3858
Elephant Supplement	90.6	2.33	0	976870	2972828	643115	0.0388	200	17638	3790
Elephant Supplement	92.1	3.85	0	747316	1792552	400159	0.0276	200	19544	4333
Elephant Supplement	93.5	5.06								
ADF 16	90.8	3.16	0	1031497	1721935	347165	0.0331	200	11329	2269
ADF 16	92.2	3.50	0	648986	1523954	285604	0.0451	200	11696	2177
ADF 16	93.5	2.67								
ADF 16	90.7	2.88	0	785827	1551261	340658	0.0305	200	14529	3169
ADF 16	92.1	2.13	0	695181	1335612	266562	0.0301	200	14328	2840
ADF 16	93.9	4.06								
ADF 16 Herbivorous 1/2" O.H. Kruse	91.3	2.50	0	738234	1231271	237126	0.0344	200	10860	2077

Table A.6 continued.

Type of Sample	%DM	%EE	Tannin Rating	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Wet Sample Weight (g)	Volume of ISTD Spike (µL)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
ADF 16 Herbivorous 1/2" O.H. Kruse	92.1	2.55	0	769639	1249741	273602	0.0348	200	10452	2273
ADF 16 Herbivorous 1/2" O.H. Kruse	93.9	3.80								
Elephant Diet	89.8	1.99	0	759608	504073	158896	0.0293	200	5132	1607
Elephant Diet	91.2	0.85	0	656284	466092	115515	0.0313	200	5142	1266
Elephant Diet	93.1	1.54								
Cargill ADF 16	91.6	2.30	0	694382	1683483	257013	0.0333	200	16267	2467
Cargill ADF 16	92.7	3.12	0	817683	1666150	244274	0.0296	200	15381	2240
Cargill ADF 16	93.7	3.98								
Mazuri Moose Maintenance	91.5	4.94	0	634035	2397617	472324	0.0466	200	18079	3537
Mazuri Moose Maintenance	92.8	5.91	0	799833	3290107	738179	0.0389	200	23559	5250
Mazuri Moose Maintenance	94.5	6.43								
ADF 16	88.7	3.33	0	675763	1492445	307357	0.0323	200	15660	3203
ADF 16	90.4	2.60	0	741356	1807330	453221	0.0369	200	15131	3769
ADF 16	92.1	3.49								
Textured Grain Mix	89.5	3.12	0	658076	1592764	373775	0.0318	200	17254	4021
Textured Grain Mix	91.3	3.67	0	713625	2264056	535884	0.0437	200	16458	3869
Textured Grain Mix	93.2	4.92								
Pellet	90.3	0.60	0	616565	661059	50861	0.0286	200	8532	652
Pellet	91.3	1.31	0	909792	792230	58430	0.0350	200	5663	415

Table A.6 continued.

Type of Sample	%DM	%EE	Tannin Rating	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Wet Sample Weight (g)	Volume of ISTD Spike (µL)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
Pellet	91.3	2.77								
Open Formula Herbivore Grain (specially formulated by Brookfield Zoo)	88.7	3.00	0	546439	967978	144882	0.0290	200	14011	2083
Open Formula Herbivore Grain (specially formulated by Brookfield Zoo)	90.3	2.36	0	764282	1634891	297121	0.0354	200	13860	2502
Open Formula Herbivore Grain (specially formulated by Brookfield Zoo)	91.8	4.00								
South African Browse Pellet	92.3	1.48	2	589190	1781060	239357	0.0369	200	18111	2417
South African Browse Pellet	95.0	3.58	2	534307	1529459	153162	0.0434	200	14582	1450
Mazuri ADF 25	90.5	2.23	0	522165	781334	187940	0.0292	200	11564	2763
Mazuri ADF 25	93.0	3.63	0	618243	1079199	225046	0.0340	200	11586	2400

Table A.7 Original data, including wet sample weight in grams, % dry matter, % ether extract, ISTD heptadecanoic Acid (17:0) peak area, linoleic acid (18:2n6) peak area, linolenic acid (18:3n3) peak area, and gamma linolenic acid (18:3n6) peak area, and colorimetric condensed tannin rating for the category of dried African browse. An (*) indicates that the sample was analyzed only once in the particular category due to a lack of available material.

Type of Sample	%DM	%EE	Tannin Rating	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Wet Sample Weight (g)	Volume of ISTD Spike (µL)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
Ziziphus mucronata	93.3	3.93	3	1984076	419265	1036358	0.0514	200	929	2281
Ziziphus mucronata	89.9	2.69	3	572157	78718	219642	0.0438	200	710	1968
Acacia karroo	91.9	2.73	3	1859550	451958	887165	0.0397	200	1369	2669
Acacia karroo	93.3	1.14	3	656735	135753	275259	0.0388	200	1191	2399
Securanegra virosa	91.6	5.51	1	2149674	375233	1789965	0.0370	200	1065	5047
Securanegra virosa	91.8	2.31	1	665607	124652	632058	0.0378	200	1119	5633
Grewia monticola	93.0	4.73	3	2243609	323566	1108818	0.0354	200	908	3090
Grewia monticola	92.8	4.68	3	759692	333875	383228	0.0397	200	2467	2813
Dichrostachys cineria	92.7	2.49	3	2058779	142579	771621	0.0386	200	401	2154
Dichrostachys cineria	92.7	1.24	3	687899	44436	214598	0.0359	200	402	1928
Elephantorrhiza goetzii	91.9	5.30	3	997696	148981	533544	0.0399	200	840	2989
Elephantorrhiza goetzii	92.5	6.78	3	690975	92178	441754	0.0350	200	856	4074
Dalbergia melanoxylon	93.0	2.56	2	1356201	107119	226147	0.0396	200	444	930
Dalbergia melanoxylon	93.2	0.75	2	727391	107707	269945	0.0425	200	775	1929

Table A.7 continued.

Type of Sample	%DM	%EE	Tannin Rating	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Wet Sample Weight (g)	Volume of ISTD Spike (µL)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
Schrebra trichoclada	93.2	4.52	1	1623977	0	0	0.0392	200	0	0
Schrebra trichoclada	92.3	1.64	1	799782	0	42878	0.0432	200	0	275
Diospyros quiloensis	92.5	4.97	3	1713874	165775	331202	0.0451	200	476	945
Diospyros quiloensis	93.9	4.34	3	690286	60886	0	0.0455	200	431	0
Commiphora mossambiscensis	91.8	5.65	3	1618117	203783	310842	0.0416	200	678	1028
Commiphora mossambiscensis	93.0	3.64	3	653363	92098	46174	0.0421	200	750	374
Vitex petersiana	93.2	2.61	1	655386	0	77399	0.0406	200	0	639
Vitex petersiana	94.1	1.04	1	595989	47720	89816	0.0394	200	449	840
Pterocarpus rotundifolia	93.0	2.43	2	749452	0	134066	0.0370	200	0	1062
Pterocarpus rotundifolia	94.2	0.26	2	778081	88143	243721	0.0407	200	616	1691
Grewia monticola	*92.9	*4.55	1	759637	0	46460	0.0326	200	0	415
Grewia monticola	*92.9	*4.55	1	594671	51148	0	0.0408	200	470	0
Combretum zeyheri	92.2	4.54	1	892977	0	0	0.0391	200	0	0
Combretum zeyheri	93.6	2.71	1	787081	58248	0	0.0470	200	351	0
Cassia abreviata	93.9	5.14	2	849956	0	154736	0.0377	200	0	1052
Cassia abreviata	94.8	6.00	2	629079	56526	92533	0.0414	200	476	774

Table A.8 Original data, including wet sample weight in grams, % dry matter, % ether extract, ISTD heptadecanoic Acid (17:0) peak area, linoleic acid (18:2n6) peak area, linolenic acid (18:3n3) peak area, and gamma linolenic acid (18:3n6) peak area, and colorimetric condensed tannin rating for dried North American browse which was used in the degradation study. An (*) indicates that the sample was analyzed only once in the particular category due to a lack of available material.

Type of Sample	%DM	%EE	Tannin Rating	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Wet Sample Weight (g)	Volume of ISTD Spike (µL)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
Morus alba dried stems 1995	*93.8	*2.15	0	767888	239540	109626	0.0360	200	1913	869
Morus alba dried stems 1995	*93.8	*2.15	0	643922	219398	99450	0.0405	200	1857	836
Morus alba dried stems 1994	93.2	2.03	0	884156	146056	0	0.0388	200	940	0
Morus alba dried stems 1994	94.3	1.12	0	637958	110558	0	0.0391	200	979	0
Morus alba dried leaves 1995	*92.7	*6.24	0	912319	877550	1737186	0.0374	200	5744	11294
Morus alba dried leaves 1995	*92.7	*6.24	0	594251	974077	1928077	0.0426	200	8594	16895
Morus alba dried leaves 1994	*92.3	*4.94	1	777189	67389	331893	0.0401	200	485	2373
Morus alba dried leaves 1994	*92.3	*4.94	1	649353	110469	777840	0.0581	200	657	4593

Table A.8 continued.

Type of Sample	%DM	%EE	Tannin Rating	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Wet Sample Weight (g)	Volume of ISTD Spike (µL)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
Liquidambar styraciflua	91.9	4.33	3	633631	75672	269506	0.0374	200	711	2516
Liquidambar styraciflua	94.0	1.88	3	774614	133594	336717	0.0490	200	784	1963
Lirodendron tulipifera	92.5	3.34	3	574961	174819	260565	0.0454	200	1485	2198
Lirodendron tulipifera	94.3	3.40	3	667050	159348	235578	0.0420	200	1261	1851
Morus alba	91.5	4.04	2	534284	236984	251184	0.0363	200	2735	2879
Morus alba	93.5	2.97	2	747032	171004	320696	0.0379	200	1352	2518
Populus deltoides	93.3	4.48	0	701941	93101	224652	0.0457	200	638	1528
Populus deltoides	95.2	2.69	0	922522	101767	246473	0.0371	200	653	1571
Rhus glabra	93.2	5.08	3	586089	83191	582622	0.0350	200	891	6197
Rhus glabra	95.3	4.37	3	690019	75294	606589	0.0396	200	605	4843
Vitis labrusca	90.8	3.52	3	622419	138653	305737	0.0313	200	1599	3502
Vitis labrusca	93.5	2.48	3	851113	336257	516841	0.0405	200	2192	3346
Salix babylonica	91.6	2.32	3	679369	79615	0	0.0321	200	812	0
Salix babylonica	94.5	2.53	3	872769	172856	279917	0.0456	200	966	1554
Malus coronaria	93.0	1.78	3	656042	55798	169524	0.0321	200	583	1759
Malus coronaria	95.2	2.97	3	762397	74674	246364	0.0374	200	576	1888
Platanus occidentalis	91.8	1.39	3	650850	171140	0	0.0380	200	1539	0
Platanus occidentalis	94.4	2.03	3	681692	89417	133359	0.0432	200	675	1000
Quercus rubra	92.0	2.69	3	620400	0	0	0.0372	200	0	0
Quercus rubra	94.4	2.73	3	518512	0	0	0.0432	200	0	0

Table A.9 Original data, including wet sample weight in grams, % dry matter, % ether extract, ISTD heptadecanoic Acid (17:0) peak area, linoleic acid (18:2n6) peak area, linolenic acid (18:3n3) peak area, and gamma linolenic acid (18:3n6) peak area for the category of fresh North american browse. Tannin rating was only performed on dried and ground samples. An (*) indicates that the sample was analyzed only once in the particular category due to a lack of available material.

Type of Sample	%DM	%EE	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Wet Sample Weight (g)	Volume of ISTD Spike (µL)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
Liquidambar styraciflua	30.0	3.10	6303551	331387	2571355	0.0319	400	2161	16654
Liquidambar styraciflua	30.0	3.10	5989328	319467	1209986	0.0202	400	3462	13025
Liquidambar styraciflua	30.0	3.10	6434829	121569	1996506	0.0238	400	1041	16978
Liquidambar styraciflua	30.0	3.10	6819063	447310	1288165	0.0176	400	9774	27957
Liquidambar styraciflua	30.0	3.10	6150303	242846	2682313	0.0286	400	3621	19860
Lirodendron tulipifera	29.0	3.37	6600018	204673	1907351	0.0303	400	1388	12850
Lirodendron tulipifera	29.0	3.37	6073922	245094	1697414	0.0240	400	2281	15688
Lirodendron tulipifera	29.0	3.37	5978511	313880	1807850	0.0220	400	3237	18518
Morus alba	24.0	3.51	6307759	348447	2588296	0.0237	400	3820	28186
Morus alba	24.0	3.51	6641924	352317	2929044	0.0257	400	3383	27934
Morus alba	24.0	3.51	6438114	370778	2738683	0.0238	400	3966	29097
Populus deltoides	30.0	3.59	6693575	400048	1517014	0.0333	400	2353	8864
Populus deltoides	30.0	3.59	6507010	216045	1894058	0.0241	400	1806	15730
Populus deltoides	30.0	3.59	6425913	276658	2076847	0.0304	400	1857	13846
Rhus glabra	25.0	4.72	7279599	205625	2575925	0.0150	400	2963	36867
Rhus glabra	25.0	4.72	7021834	517128	2531977	0.0171	400	6777	32955

Table A.9 continued.

Type of Sample	%DM	%EE	ISTDme Peak Area	18:2n6me Peak Area	18:3n3me Peak Area	Wet Sample Weight (g)	Volume of ISTD Spike (µL)	ppm 18:2n6 (µg/g)	ppm 18:3n3 (µg/g)
Rhus glabra	25.0	4.72	7189607	235439	3083413	0.0211	400	2442	31765
Vitis labrusca	21.0	3.00	6167904	393302	1324755	0.0205	400	5827	19493
Vitis labrusca	21.0	3.00	6527826	459618	2313690	0.0271	400	4867	24333
Vitis labrusca	21.0	3.00	6686515	458153	1899717	0.0236	400	5439	22398
Salix babylonica	30.0	2.42	6893009	508510	2751949	0.0346	400	2796	15027
Salix babylonica	30.0	2.42	6939082	431609	1158501	0.0171	400	4770	12715
Salix babylonica	30.0	2.42	6984556	436180	1149972	0.0202	400	4054	10615
Malus coronaria	40.0	2.37	6705919	285461	3190923	0.0344	400	1217	13511
Malus coronaria	40.0	2.37	6775337	169123	1907799	0.0248	400	990	11090
Malus coronaria	40.0	2.37	6386039	139761	2497963	0.0266	400	809	14364
Platanus occidentalis	28.0	1.71	8323017	174756	1627454	0.0163	400	1810	16739
Platanus occidentalis	28.0	1.71	6831812	137849	1343088	0.0185	400	1532	14828
Platanus occidentalis	28.0	1.71	6449709	380473	1792763	0.0263	400	3151	14747
Quercus rubra	41.0	2.71	3321826	288372	2203939	0.0253	200	1646	12495
Quercus rubra	41.0	2.71	3442325	264863	1723919	0.0196	200	1883	12174
Quercus rubra	41.0	2.71	4272634	367490	2298928	0.0243	200	1698	10550

Table A.10 Breakdown of black rhinoceros diets by percent of the rhino's total diet found in each category.

Rhinoceros Holding Facility	Alfalfa Hay	Pellet	Fresh Browse	Produce	Grass Based Hay	Daily Intake (kg)
Brookfield Zoo	70	27	0	3	0	41
Busch Gardens	21	71	4	4	0	25
Cincinnati Zoo	55	37	0	7	0	37
Dallas Zoo	64	26	6	4	0	43
Denver Zoological Foundation	70	12	2	6	10	22
Denver Zoological Foundation	73	10	2	5	9	25
Denver Zoological Foundation	62	16	2	12	8	11
Detroit Zoological Park	45	29	8	16	0	28
Fossil Rim Wildlife Center	24	37	2	17	20	46
Fossil Rim Wildlife Center	29	35	2	9	24	39
Fossil Rim Wildlife Center	28	50	4	18	0	49
Lee Richardson Zoo	44	18	2	2	35	30
Los Angeles Zoo	51	12	20	16	0	22
Miami Metrozoo	36	15	10	3	36	30
Miami Metrozoo	36	15	10	3	36	30
Miami Metrozoo	0	20	15	5	60	18
Miami Metrozoo	14	28	10	7	41	13
Miami Metrozoo	14	28	10	7	41	13
Milwaukee County Zoo	32	26	4	6	32	23
Oklahoma City Zoological Park	35	39	4	4	17	21
Riverbanks Zoological Park and Botanical Gardens	68	27	3	1	0	40

Table A.10 continued.

Rhinoceros Holding Facility	Alfalfa Hay	Pellet	Fresh Browse	Produce	Grass Based Hay	Daily Intake (kg)
Sedgewick County Zoo	36	44	0	2	18	25
Sedgewick County Zoo	85	13	0	2	0	26
White Oaks Conservation Center	19	24	10	7	39	19
Wildlife Conservation Society	0	13	3	13	71	15
Zoo Atlanta	33	28	2	15	21	28
Zoo Atlanta	37	17	3	17	24	28

Table A.11 Summary table of percent dry matter (%DM) and percent ether extract (%EE) of all classes of of black rhinoceros feeds.

SAMPLE TYPE	n	% DM ± Std. Dev.	% EE ± Std. Dev.
Alfalfa Hay	18	91.6 ± 1.8	1.9 ± 0.6
Pellets	26	91.5 ± 0.9	3.0 ± 0.9
Grass Based Hay	13	92.7 ± 0.9	1.8 ± 0.7
Fresh North American Browse	10	29.8 ± 6.4	3.1 ± 0.8
African Browses	14	92.8 ± 0.7	3.4 ± 1.5
Composite North American Captive Diet	N/A	81.4	2.1

Table A.12 Linoleic Acid (18:2n6) in all classes of black rhinoceros feeds.

Item	n	% 18:2 of Total Lipids ± Std. Dev.	Range
Alfalfa Hay	18	7 ± 3.0	4 - 12
Pellets	26	46 ± 9	29 - 62
Grass Based Hay	13	6 ± 3	2 - 11
Fresh North American Browse	10	5 ± 4	3 - 32
African Browses	14	2 ± 2	0 - 7
Composite North American Captive Diet		16	

Table A.13 Alpha Linolenic acid in all classes of black rhinoceros feeds.

Item	n	% 18:3n3 of Total Lipids ± Std. Dev.	Range
Alfalfa Hay	18	12 ± 7	5 - 23
Pellets	26	9 ± 2	1 - 13
Grass Based Hays	13	13 ± 8	0 - 29
Fresh North American Browse	10	61 ± 18	25 - 90
African Browses	14	6 ± 5	0 - 14
Composite North American Captive Diet		13	

Table A.14 Estimate of each feed category's contribution to total Linoleic acid in diets of North American captive black rhinoceroses, dried African browse and fresh North American browse. The sum of the first four categories was used to determine the % of linoleic acid of total lipids in the composite diet.

Feed Category	Estimated % of Diet	% EFA of Total Lipid	Contribution towards % 18:2n6 of Total Lipid
Alfalfa Hay	41	7	3
Pellets	26	46	12
Grass Based Hay	20	6	1
Fresh North American Browses	5	5	0.3
Produce	8	Not Evaluated	Not Evaluated
Composite North American Captive Diet	N/A	2	16
African Browses	100	2	2
Fresh North American Browses Only	100	5	5

Table A.15 Estimate of each feed category's contribution to total α-Linolenic acid in diets of North American captive black rhinoceroses, dried African browse and fresh North American browse. The sum of the first four categories was used to determine the % of linolenic acid of total lipids in the composite diet.

Feed Category	Estimated % of Diet	% EFA of Total Lipid	Contribution towards % 18:3n3 of Total Lipid
Alfalfa Hay	41	12	5
Pellets	26	9	2
Grass Based Hay	20	13	3
Fresh North American Browses	5	61	3
Produce	8	Not Evaluated	Not Evaluated
Composite North American Captive Diet	N/A	N/A	13
African Browses	100	6	6
Fresh North American Browses Only	100	61	61

Table A.16 Degradation of Linoleic acid (18:2n6) in ten fresh North American browses over an approximately 140 day period.

Browses at Day 0	n	% 18:2n6 of Total Lipid ± Std. Dev.	Range
Liquidambar styraciflua	5	13 ± 9.8	3 - 32
Lirodendron tulipifera	3	7 ± 2.2	4 - 10
Morus alba	3	11 ± 0.7	10 - 11
Populus deltoides	3	6 ± 0.7	5 - 7
Rhus glabra	3	9 ± 4.1	5 - 14
Vitis labrusca	3	18 ± 1.3	16 - 19
Salix babylonica	3	16 ± 3.4	12 - 20
Malus coronaria	3	4 ± 0.7	3 - 5
Platanus occidentalis	3	13 ± 4.1	9 - 18
Quercus rubra	3	6 ± 0.4	6 - 7
Browses at Approximately Day 140	n	% 18:2n6 of Total Lipid ± Std. Dev.	Range
Liquidambar styraciflua	2	4 ± 1.4	2 - 5
Lirodendron tulipifera	2	6 ± 1.5	4 - 7
Morus alba	2	10 ± 5.9	4 - 16
Populus deltoides	2	3 ± 0.9	2 - 4
Rhus glabra	2	2± 1.2	1 - 4
Vitis labrusca	2	10 ± 4.6	5 - 14
Salix babylonica	2	5 ± 1.4	4 - 7
Malus coronaria	2	4 ± 1.2	2 - 5
Platanus occidentalis	2	11 ± 7.0	4 - 18
Quercus rubra	2	0 ± 0.0	0 - 0
Species		Loss of % 18:2n6 ± Std. Dev.	Range
Liquidambar styraciflua		52 ± 33.5	-9 - 88
Lirodendron tulipifera		2 ± 42.0	-44 - 38
Morus alba		7 ± 7.8	-2 - 13
Populus deltoides		51 ± 6.8	46 - 59
Rhus glabra		58 ± 22.0	40 - 82
Vitis labrusca		45 ± 5.1	39 - 49
Salix babylonica		65 ± 10.0	54 - 73
Malus coronaria		11 ± 18.1	-8 - 28
Platanus occidentalis		5 ± 32.1	-22 - 4
Quercus rubra		100 ± 0.0	100 - 100
Total Loss		40	-44 - 100

Table A.17 Degradation of α-Linolenic acid in fresh North American browses over an approximately 140 day period.

Browses at Day 0	n	% 18:3n3 of Total Lipid ± Std. Dev.	Range
Liquidambar styraciflua	5	61 ± 18	42 - 90
Lirodendron tulipifera	3	46 ± 6.9	38 - 55
Morus alba	3	81 ± 1.4	80 - 83
Populus deltoides	3	36 ± 8.1	25 - 44
Rhus glabra	3	72 ± 4.6	67 - 78
Vitis labrusca	3	74 ± 6.6	65 - 81
Salix babylonica	3	53 ± 7.4	44 - 62
Malus coronaria	3	55 ± 5.8	47 - 61
Platanus occidentalis	3	90 ± 5.4	86 - 98
Quercus rubra	3	43 ± 3.1	39 - 46
Browses at Approximately Day 140	n	% 18:3n3 of Total Lipid ± Std. Dev.	Range
Liquidambar styraciflua	2	7 ± 0.8	6 - 8
Lirodendron tulipifera	2	6 ± 0.5	5 - 6
Morus alba	2	7 ± 0.5	7 - 8
Populus deltoides	2	4 ± 0.1	4 - 4
Rhus glabra	2	11 ± 1.4	10 - 12
Vitis labrusca	2	11 ± 0.2	10 - 11
Salix babylonica	2	3 ± 3.1	0 - 6
Malus coronaria	2	7 ± 0.3	7 - 8
Platanus occidentalis	2	3 ± 2.8	0 - 6
Quercus rubra	2	0 ± 0.0	0 - 0
Species		Loss of % 18:3n3 ± Std. Dev.	Range
Liquidambar styraciflua		89 ± 3.7	84 - 94
Lirodendron tulipifera		88 ± 2.3	85 - 90
Morus alba		91 ± 0.2	91 - 91
Populus deltoides		88 ± 3.9	83 - 91
Rhus glabra		84 ± 0.3	84 - 84
Vitis labrusca		85 ± 1.7	84 - 87
Salix babylonica		94 ± 1.0	93 - 95
Malus coronaria		86 ± 1.9	84 - 88
Platanus occidentalis		97 ± 0.2	97 - 97
Quercus rubra		100 ± 0.0	100 - 100
Total Loss		90	83 - 100

Table A.18 Average condensed tannin rating of items and browses preferred by black rhinoceroses.

Feed Category	Average Condensed Tannin Rating
Alfalfa Hay	0.0
Pellets	0.0
Grass Based Hay	0.5
North American Browses	1.9
Produce	Not Evaluated
African Browses	2.1
Composite North American Captive Diet	0.2

Table A.19 A list of produce fed to captive North American black rhinos.

Type of Produce	Number of Facilities Offering Produce
Apple	12
Carrot	12
Sweet Potato/Yam	5
Banana	5
Orange	2
Onion	2
Lettuce	1
Pineapple	1
Potato	1
Pear	2
Spinach	1
Celery	1
Winter Squash	1
Green Beans	1

Table A.20 A list of fresh browses fed to captive North American black rhinos.

Type of Browse	Number of Facilities Offering Browse
Hibiscus (*Hibiscus rosasinensis*)	2
Banana Leaves (*Musa paradisiaca*)	3
Bamboo Stems (*Phyllostochys*)	5
Honeysuckle	1
Mulberry	4
Hackberry	1
Other Species	1
Crabapple	1
Cottonwood	1
Elm	1
Ash	1
Honey Locust	1
Sumac (*Rhus* spp.)	1
Willow (*Salix* spp.)	3
Mesquite (*Prospsis juliflora*)	1
Spectrum Leafeater	1
Black Acacia	1
Purple Orchid Tree (*Bauhinia purpurea*)	1
Hong Kong Orchid Tree (*Bauhinia blakeana*)	1
Black Olive (*Bucida buceras*)	1
Ficus (*Ficus benjamina*)	1
Benjamin Fig	1
Weeping Fig	1
Privet (*Ligustrum japonicum*)	1
Cane grass (*Panicum hemitomon*)	1
Sugar Cane (*Saccharum officinalum*)	1
Scheffelera	1
Dwarf Scheffelera	1
Sugar Maple (*Acer saccharum*)	1
Silver Maple (*Acer saccharinum*)	1
Oak (*Quercus* spp.)	1
Cane	1
Wax Myrtle (*Myrica cerifera*)	1
Sassafras (*Sassafras albidum*)	1
Yellow Poplar (*Lirodendron tulipifera*)	1
Sweetgum (*Liquidambar styraciflua*)	2

Table A.20

Type of Browse	Number of Facilities Offering Browse
American Sycamore (*Plantanus occidentalis*)	1
Salt Bush (*Baccharis halimifolia*)	1
Bay (*Laurus nobilis*)	1
Tupelo (*Nyssa sylvatica*)	1
Acacia app.	1
Pine spp.	1

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