THE CAPTIVE MANED WOLF (*Chrysocyon brachyurus*): NUTRITIONAL CONSIDERATIONS WITH EMPHASIS ON MANAGEMENT OF CYSTINURIA

By

Sara E. Childs-Sanford

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Advisory Committee

Dr. Roselina Angel, Chair
Dr. Brian Bequette
Dr. Joseph Soares

ABSTRACT

Title of Thesis: THE CAPTIVE MANED WOLF (*Chrysocyon brachyurus)*: NUTRITIONAL CONSIDERATIONS WITH EMPHASIS ON MANAGEMENT OF CYSTINURIA

Degree Candidate: Sara E. Childs-Sanford
Master of Science, 2005

Thesis directed by: Dr. Roselina Angel
Department of Animal and Avian Sciences

The intent of this project was to investigate options for improvement of a commercially manufactured prescription diet designed to reduce manifestation of clinical disease secondary to cystinuria in captive maned wolves in the United States. Diets high in plant-based protein, independent of sulfur amino acid content, resulted in an increase in average urine pH as well as a decrease in urine cystine crystal formation and cystine excretion in maned wolves. The rate of passage of digesta in maned wolves was very similar to that of domestic dogs on either the commercially available formula and an experimental formula. Differences in nutrient digestibility and mineral retention were seen between the wolves and the dogs, with dogs exhibiting higher digesitibility or retention in all cases. Six maned wolves maintained on these same two diets exhibited plasma taurine concentrations markedly lower than canine and feline normal reference ranges, implying that maned wolves may have a dietary requirement for taurine.

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CHAPTER 1: Literature Review

1.1 The Wild Maned Wolf

The maned wolf, *Chrysocyon brachyurus*, inhabits the grasslands, savannahs, swamplands, and scrub forests of central and eastern Brazil, northern Argentina, Paraguay, eastern Bolivia, and southeastern Peru (Langguth 1975, Dietz 1984). It is hypothesized that, like other South American canids, the maned wolf evolved from a common canid ancestor that crossed the Panama landbridge during the early Pliocene era (Langguth 1975). Many features distinguish the maned wolf from its canid relatives, justifying its classification in a separate genus, *Chrysocyon*, of which it is the sole member. For instance, the diploid chromosome number of *Chrysocyon* (78) differs from that of *Canis* (76) (Newnham and Davidson 1966). Striking physical characteristics such as long slender legs and large erect pinnae are likely anatomical adaptations to facilitate locomotion and capturing of prey in the tall grasses of their natural habitat (Kleiman 1972, Dietz 1984). Their name is derived from the presence of long black erectile hairs along the dorsal cervical and thoracic regions. The activities of the maned wolf are primarily crepuscular. Maned wolves are solitary animals, although mating pairs are often seen together during the breeding season. Single animals or mated pairs typically defend large territories, averaging 20-30 square kilometers (Dietz 1984).

The maned wolf does not prey upon large vertebrates as is typical of most other wolf species. Instead, it has been found to be an opportunistic omnivore, taking advantage of smaller food items that are seasonally available in its natural habitat. Based on scat analysis, Motta-Junior et al. (1996) estimated that 51% of the wild

maned wolf diet is composed of plant matter, while 49% is derived from animal matter. The animal portion, which increases during the dry season, consists primarily of small mammals such as rodents and armadillos, as well as birds, reptiles, amphibians, and some invertebrates (Dietz 1984, Dietz 1987, Motta-Junior et al. 1996). The plant portion, most abundant during the wet season, is composed largely of various fruits. One fruit in particular, *Solanum lycocarpum*, the availability of which is year-round, is consumed in large quantities by the maned wolf (Dietz 1984, Dietz 1987, Motta-Junior et al. 1996). This tomato-like fruit grows on a shrub and is eaten by the wolf in both ripe and unripe stages (Dietz 1984, Motta-Junior et al. 1996). The strong dependence of the maned wolf on this fruit is indicated by the large percentage of it in analyzed scats as well as by the common name for this fruit in certain regions of Brazil, "lobiera", which means "fruit of the maned wolf".

The maned wolf is classified as “near-threatened” on the 2004 International Union for the Conservation of Natural Resources (IUCN) Red List, as endangered by the US Fish and Wildlife Service, and is listed under Appendix II of the Convention on International Trade in Endangered Species (CITES). This reclusive wolf avoids contact with humans at all costs, and its decline in the wild is primarily a result of habitat destruction for agricultural development. Encroachment of humans on the habitat of the maned wolf results in fragmentation of territorial ranges and reduction of available food sources. Ranchers occasionally hunt maned wolves as retribution for killed domestic livestock. Although unable to prey upon larger livestock species, maned wolves are known to have a fondness for domestic chickens (Dietz 1984). Furthermore, ancient folklore contributes to their decline by propagating false notions

that certain anatomical parts of the maned wolf have magical qualities. For example, the right eye removed from a live maned wolf is said to be a good luck charm and to increase sexual prowess. A canine tooth is said to protect children who wear one around their neck from dental problems, and ingestion of cardiac muscle from a recently killed wolf supposedly saves a snake-bite victim from death (Dietz 1984).

1.2 The Captive Maned Wolf

There is a relatively small captive population of maned wolves around the world. There are currently less than one-hundred maned wolves maintained in zoological parks across the United States, and all are managed under a Species Survival Plan (M. Rodden, personal communication, 2001). In captivity, the maned wolf has historically been fed like a carnivore, with early diets being composed entirely of raw meat. Subsequent diets were primarily formulated and manufactured for felids, with a switch in more recent years to diets formulated for canids (Bush 1980, Allen et al. 1996).

For much of its history in captivity, the maned wolf has been known to be generally unthrifty and have marginal reproductive success (Brady and Ditton 1978). Relatively poor body condition, extremely rapid passage of digesta, chronically soft stools, and gingivitis (Brady and Ditton 1978, Bush 1980) are conditions that are being or have been remedied with husbandry changes based primarily on increased knowledge about the natural history of these animals. One serious and widely recognized medical condition afflicting captive maned wolves with an alarming

frequency is cystinuria, characterized by excess levels of the amino acid cystine in the urine.

1.3 Cystinuria

Cystinuria is a disorder which affects both humans and domestic dogs, and in these species, it has been proven to be a genetically inherited metabolic defect (Harris et al. 1955, Tsan et al. 1972, Goodyer et al. 2000). This autosomal recessive disorder is characterized by aberrant transport of cystine and the dibasic amino acids lysine, ornithine, and arginine through the epithelial cells of both the renal tubular and intestinal brush borders (Harris et al. 1955, Rosenberg et al. 1965, Casal et al. 1995). The disorder is common in humans, with a prevalence of approximately 1 in 7,000-15,000, although in certain ethnic groups or geographic regions, the prevalence is as high as 1 in 2500 (Weinberger et al. 1974, Goodyer et al. 2000, Guillén et al. 2004). The prevalence in dogs varies with geographic region and breed, being much higher in most European countries than the United States, and being most frequently reported in Dachshunds, English Bulldogs, Mastiffs, Basset Hounds, and Newfoundlands (Case et al. 1992, Bartges et al. 1993, Osborne et al. 1999).

The clinical manifestations of cystinuria develop secondary to the formation of cystine uroliths, which, depending on their location, can cause life-threatening upper or lower urinary tract obstruction. The presence of uroliths can also predispose the patient to complications such as urinary tract infections and renal insufficiency (Lindell et al. 1997, Rutchik and Resnick 1997, Joly et al., 1999, Goodyer et al. 2000). Cystinuria is reported to account for 1-2% of nephroliths observed in human

adults and 6-8% percent of those in children (Milliner and Murphy 1993, Gregory and Schwartz 1998, Dell and Guay-Woodford 1999, Escolar and Bellanato 1999, Joly et al. 1999). In European countries, cystine calculi have been reported to be the cause of 15-40% of the uroliths in domestic dogs, while in the United States, this value is only 2.4-3.8% (Osborne et al. 1986, Case et al. 1992).

Although cystine, ornithine, lysine, and arginine are all excreted in excess in cystinuria, only cystine is involved in crystal and urolith formation. Cystine is the least soluble of these four amino acids, and crystallizes in supersaturated urine. Furthermore, the solubility of cystine is highly dependent on pH (Osborne et al. 1997). The pKa of cystine is 8.3, making it relatively insoluble at normal urine pHs of 5 to 7. As the urine becomes more acidic, the solubility decreases rapidly, and cystine precipitates into crystal form (Treacher 1966, Osborne et al. 1989b). The solubility of cystine therefore increases with increasing urinary pH, almost doubling at pH 7.8 and tripling at pH 8.0 (Dent and Senior 1995). Conversely, lysine, the excretion of which commonly exceeds that of cystine in cystinuria, is completely water soluble, as are the other dibasic amino acids (Ng and Streem 1999). Although excretion of large amounts of the essential amino acids lysine and arginine occurs, nutritional deficiencies do not appear to develop (Hellier et al. 1970, Silk 1974). The explanation for this phenomenon may be due in part to the fact that the jejunal transport defect for these amino acids affects only monomers, while their intestinal absorption in dipeptide form is not impaired (Hellier et al. 1970, Silk 1974).

1.3.1 *Normal Physiology*

Due to their relatively small molecular weight, plasma amino acids pass freely through the glomerular membrane in the renal cortex, and the amino acid concentration within the glomerular filtrate is approximately equal to that in the plasma (Guyton and Hall, 1996). In order to avoid the loss of these important nutrients in the urine, they are reabsorbed in the renal tubules. This reabsorption is very efficient, with between 95 and 100 percent of the filtered amino acid load being returned to the plasma (Hoppe 1994). The main site of reabsorption is in the proximal tubules, where amino acids undergo secondary active transport across the tubular epithelial cells. The cells lining the proximal tubules are capable of this high transport load due to several specialized characteristics including large numbers of mitochondria, an extensive luminal brush border, and an intricate labyrinth of intercellular and basal channels (Guyton and Hall 1996).

The most common basic mechanism of free amino acid transport across the proximal renal tubular and intestinal epithelial cells is via cotransport with sodium (Guyton and Hall 1996). The amino acids are coupled with specific carriers that require sodium binding, and the amino acids diffuse into the epithelial cells as sodium is transported intracellularly. An electrochemical gradient in favor of sodium absorption from the renal tubular and intestinal lumens is created by a sodium-potassium ATPase located at the basal side of the membrane. Sodium is actively transported from the epithelial cells into the peritubular and mesenteric capillaries by the sodium-potassium pump, while specific carriers are present for transport of amino acids extracellularly at the basal membrane and into the bloodstream.

1.3.2 *Cystinuria Physiology and Molecular Genetics*

Amino acid carriers have been identified which are specific for amino acids within a certain group. Cystine and the dibasic amino acids ornithine, lysine, and arginine, have been demonstrated to all use the same transporter system in the intestinal and renal tubular epithelial cells. This system functions independent of sodium, and consists of a heterodimeric transporter composed of a heavy and light subunit that are covalently associated and are members of the $b^{o,+}$ transport system (Dell and Guay-Woodford 1999, Pfieffer et al. 1999, Palacin et al. 2000). The heavy subunit is the protein rBAT, and the recently identified light subunit is the protein $b^{o,+}$AT (Palacin et al. 2000). Two different reabsorption systems have been identified in the apical membrane of the proximal tubule cells. There is both a high-affinity transport mechanism which transports cystine and the dibasic amino acids, and a low-affinity mechanism, which transports cystine exclusively (Segal et al. 1977). The rBAT/$b^{o,+}$AT heterodimer is responsible for the high-affinity reabsorption system in both the proximal tubule and small intestine (Palacin et al., 2000). This transporter is proposed to act through an active tertiary transport mechanism which is linked to a high intracellular concentration of neutral amino acids (Goodyer et al. 2000, Palacin et al. 2000). The entry of the three dibasic amino acids would be favored by the membrane potential, while the entry of cystine from the tubular lumen would be favored by the intracellular reduction of cystine to cysteine (Palacin et al. 2000). The high concentration of intracellular neutral amino acids is obtained by apically and basolaterally located neutral amino acid transporters which are driven by the electrochemical sodium gradient established by the sodium-potassium ATPase

(Palacin et al. 2000). The export of the dibasic amino acids into the peritubular capillaries at the basolateral membrane is thought to be accomplished by the 4F2hc-y-LAT1 heterodimeric transporter (Pfeiffer et al. 1999, Palacin et al. 2000).

Rosenberg et al. (1965, 1966) originally described three cystinuria phenotypes in humans based on variations in urinary cystine excretion and intestinal amino acid transport. Type I cystinuria included heterozygotes with normal urinary amino acid excretion and homozygotes with defective intestinal transport of both cystine and the dibasic amino acids. Type II cystinuria was represented by heterozygotes with elevated urinary cystine excretion and homozygotes with normal intestinal cystine transport but impaired intestinal transport of the dibasic amino acids. Type III cystinuria included heterozygotes with abnormal urinary cystine excretion at lower levels than those in type II, as well as homozygotes with normal intestinal amino acid transport.

For decades, cystinuria was considered to be a genetically homogeneous disorder, with the three phenotypes (types I, II, and III) stemming from allelism of the same gene (Bisceglia et al. 1997). This gene, SLC3A1, which encodes the protein rBAT, was localized to the human chromosome 2p (Pras et al. 1994). Over 40 cystinuria-specific mutations have been identified in this gene, including missense, nonsense, splice site, and frameshift mutation (Palacin et al. 2000). More recently, data has been generated supporting the existence of a second cystinuria locus on the long arm of chromosome 19q13.1, which is thought to encode for the light subunit of the heterodimer, $b^{o,+}AT$, and is designated SLC7A9 (Goodyer et al. 2000, Palacin et al. 2000). Mutations in the SLC3A1 gene resulted in only the type I phenotype. On

the other hand, mutations in the SLC7A9 gene were demonstrated to account for phenotypes II and III. Thus, the phenotypic categories for human cystinuria have been redesignated by some as type I and non-type I based on recently performed genotypic studies (Pfeiffer et al. 1999, Goodyer et al. 2000, Palacin et al. 2000). Regardless of phenotypic classifications, however, most investigators agree that classification of cystinuria is highly complex, and its phenotypic expression is determined by multiple genetic and environmental factors that are not yet well understood. Future research in humans will likely continue to explore the possibility of additional cystinuria genetic defects that have not yet been identified.

1.3.3 *Diagnosis*

The identification of flat, colorless, hexagonal cystine crystals in a urine sediment is strongly suggestive of cystinuria, however this finding is present only in a minority of both human and canine patients (Rutchik and Resnick 1997, Osborne et al. 1999). In alkaline urine samples, crystal formation may be promoted by refrigeration and acidifying the urine to a pH less than 7.0 with hydrochloric or acetic acid (Gregory and Schwartz 1998, Osborne et al. 1999).

When large enough, cystine uroliths are detectable by survey radiography, and typically exhibit a radiodensity similar to that of struvite (Osborne et al. 1999). For enhanced sensitivity, especially with small uroliths, double contrast cystography is ideal, and increases the sensitivity above most techniques of ultrasonography.

The sodium-cyanide nitroprusside test is able to detect urinary cystine levels as low as 75 to 125mg/g of creatinine (Rutchik and Resnick 1997). The addition of

sodium cyanide to the urine sample results in the reduction of cystine to cysteine with the subsequent reaction of the exposed free sulfhydryl groups with nitroprusside, resulting in the formation of a red-purple color. False positive results are possible in patients on sulfur-containing drugs.

Quantitative analysis of uroliths provides a definitive diagnosis of cystinuria, however in the absence of urolithiasis, a clinical diagnosis can be made by urinary cystine quantitation. The upper limit of cystine solubility in human urine is 33mg/L, and normal humans are reported to excrete 20mg or less of cystine in their urine daily (Rutchik and Resnick 1997), however, there are numerous discrepancies between reported normal and abnormal urinary cystine levels in both humans and dogs (Hoppe 1994, Stoller et al. 1997). In one study, 7 out of 24 dogs with a history of cystine urolithiasis demonstrated urine cystine levels within the normal reference range reported for dogs (Hoppe et al. 1993). Therefore, the finding of a urinary cystine value within the reported normal range on a single sample does not exclude the possibility of cystinuria in that patient. Suspicions of cystinuria should ideally be confirmed by urine cystine quantification over a 24-hour period (Rutchik and Resnick 1997, Ng and Streem 1999, Osborne et al. 1999).

1.3.4 *Medical Management*

Medical management and prevention of clinical disease in both human and canine cystinuria revolves around the two main goals of increasing the urinary solubility of cystine as well as reducing urinary cystine concentration. These objectives are typically accomplished through a combination of dietary modification,

urinary alkalinization, and the administration of thiol-containing drugs (Joly et al. 1999, Ng and Stream 1999, Barbey et al. 2000).

Dietary manipulations are implemented to both increase urinary cystine solubility and decrease cystine excretion. The three approaches that can be utilized to achieve these results are hyperdiuresis, reduction of sodium intake, and a reduction in dietary protein. By promoting the formation of dilute urine, hyperdiuresis decreases urine cystine concentration and therefore decreases the likelihood of cystine crystallization. Although proven in most cases to be very successful, this aspect of human cystinuria is often a source of poor patient compliance. A fluid intake of 3-4 liters per day is often required, and some authors also recommend high fluid intakes at bedtime as well as during the night to prevent nocturnal supersaturation of urinary cystine (Streem 1993, Gregory and Schwartz 1998, Ng and Streem 1999). Furthermore, this aspect of dietary modification is unrealistic in veterinary patients.

The urinary excretion of cystine has been demonstrated to be directly correlated with urinary sodium excretion, and low sodium diets effectively reduced urinary cystine excretion (Jaeger et al. 1986, Peces et al. 1991, Rodriguez et al. 1995). The mechanism of this effect was originally thought to correlate with the reabsorption of urinary amino acids by a sodium-dependent mechanism. Since a sodium-independent mechanism of reabsorption of cystine and the dibasic amino acids has more recently been elucidated, the mechanism of reduced dietary sodium resulting in lower cystine excretion is not known. Patient compliance is also challenged in this situation by the decreased palatability of low sodium diets. Reduction of dietary protein intake, especially protein of animal origin, has been demonstrated to reduce

urinary cystine excretion (Osborne et al. 1989a). This is, in part, accomplished by restricting the intake of methionine, a precursor of cystine, which is especially high in animal proteins (Kolb et al. 1967, Osborne et al. 1987).

Furthermore, an alteration in the type of dietary protein might also have an effect on urinary cystine solubility through an alteration of urine pH. Urine pH is a manifestation of the overall acid base balance of the animal. Under normal physiologic conditions, the pH of mammalian urine is determined nearly entirely by dietary constituents. For maintenance of homeostasis, the hydrogen ion concentration of the blood must be restricted to a very narrow range. The kidneys play a major role in adjusting hydrogen ion concentrations of the extracellular fluid by excreting acidic or alkaline compounds at varying rates. Nonvolatile acids, produced mainly by the metabolism of proteins, are removed from the body via renal excretion. Dietary contributions from proteins to the daily acid load are derived primarily from the intake of sulfur-containing amino acids such as methionine and cysteine. Animal proteins, due to their higher levels of sulfur-containing amino acids, are more acidifying than plant proteins when consumed (Halperin 1983, Swenson 1993, Burkholder 2000), and therefore, carnivores typically have low urinary pHs while herbivores excrete a more alkaline urine. For example, the average urinary pH of the domestic cat, a true carnivore, is between 6.1 and 6.4 (Allen et al. 1997), while that of a domestic ruminant, an herbivore, is typically greater than 7.4 (Divers and VanMetre 1996). Alkalinization of the urine to increase cystine solubility can also be achieved through pharmacological means. Although sodium bicarbonate has been used,

potassium citrate has been advocated due to the effects of sodium on cystine excretion (Chow and Streem 1996).

Pharmacologic agents, such as D-penicillamine and mercaptopropionylglycine, which act by a thiol-disulfide exchange reaction, have had limited success in the long-term management of cystinuria primarily due to a high incidence of undesirable side effects (Halperin et al. 1981, Joly et al. 1999, Barbey et al. 2000). These and other agents are typically reserved for patients responding suboptimally to other forms of therapy.

1.3.5 *Cystinuria in the Maned Wolf*

Maned wolves in captivity have historically been plagued by complications related to cystinuria such as cystic and renal calculi, which cause significant morbidity and mortality (Bovee and Bush 1978, Bush and Bovee 1978, Bovee et al. 1981, Mussart and Coppo 1999). Although few studies have been performed to clearly characterize the prevalence of cystinuria in both captive and wild maned wolves, one report described abnormally elevated cystine and dibasic amino acid excretion in approximately 80% of forty-two wolves tested (Bovee et al. 1981). Of the eight wild wolves tested, six were found to be positive for cystinuria. The researchers used paper chromatography to characterize amino acid excretion, and concluded, based on their findings, that the incidence of cystinuria is extremely high in both captive and wild maned wolves, despite their marked differences in diet and habitat. This conclusion is misleading for several reasons. Firstly, the sample size of the study, especially of wild wolves, is extremely low. Furthermore, the testing of

animals from the same geographical region may have significantly biased the conclusion of a high prevalence in all wild populations. Moreover, it was stated that all captive wolves tested in the study were either directly obtained from or descended from wolves obtained from the same geographical region that all wild wolves tested were found. And finally, although it was stated that the incidence of cystinuria in both wild and captive maned wolves is high, to date, no research has been done to characterize the incidence of clinical disease related to cystinuria in wild maned wolves.

It is hypothesized that many of the clinical problems commonly seen in captive maned wolves as listed previously are a result of improper husbandry conditions, especially nutrition. For instance, the average urine pH of captive maned wolves is approximately 5.5-6.5, typical of a carnivore, and reflecting a diet that is primarily meat-based. This low urine pH drastically decreases the solubility of any cystine that may be present, predisposing wolves with underlying cystinuria to clinical complications. An additional predisposition to obstructive urolithiasis in the male maned wolf was proposed by one researcher. It was reported that the urethral diameter of male maned wolves diagnosed with cystinuria was much less than that of a domestic dog of similar size (Bush and Bovee, 1978, Bovee et al. 1981). Much more research would be needed to substantiate this claim, however. A controlled study including measurements of static and dynamic urethral diameter would be needed, especially since animals experiencing chronic urolithiasis and crystalluria from cystinuria or other similar conditions may exhibit inflammation, swelling, and even fibrosis of the urethra, which may ultimately decrease urethral luminal diameter.

There is unfortunately an extreme paucity of knowledge concerning cystinuria in the maned wolf. Several possible explanations can be considered in an effort to illuminate and illustrate the basis of cystinuria in the maned wolf. Four of these potential scenarios are presented and discussed below.

(1) Cystinuria is a genetic defect in maned wolves, has a high prevalence in both captive and wild populations, and results in a similar incidence of morbidity and mortality in both captive and wild populations.

(2) Cystinuria is a genetic defect in maned wolves, with a high prevalence in both captive and wild populations. It results in morbidity and mortality in captive maned wolves, but causes a very low or no incidence of clinical disease in wild populations.

(3) Cystinuria is a genetic defect in maned wolves, and has a high prevalence only in captive populations. The prevalence in the wild population as a whole is low, although prevalence may be higher in groups located in certain geographical regions.

(4) Cystinuria is not a genetic defect in maned wolves. It is a normal physiological phenomenon in this species for unexplained reasons. Clinical disease from this phenomenon is precipitated in a captive setting by environmental factors.

As mentioned previously, although an extremely high prevalence of cystinuria has been demonstrated in captive maned wolves, it is certainly questionable whether the finding of a high prevalence in a small number of wild wolves from the same geographical region is representative of the entire wild maned wolf population. Furthermore, the incidence of clinical disease secondary to cystinuria in wild wolves is completely unknown. It is possible that wild wolves succumb to the same levels of morbidity and mortality from cystinuria as captive wolves. On the other hand, as portrayed by the second scenario, one can also speculate that there may be fewer clinical symptoms related to cystinuria in wild wolves, despite a high prevalence of the disorder, due to an unidentified evolutionary adaptation or environmental factor. As discussed in prior sections, nutritional factors between wild and captive maned wolves differ considerably. Although we are making efforts to better approximate the omnivorous quality of the wild diet, the nutrient composition of the captive diet may still differ dramatically. For instance, the fruit of *Solanum lycocarpum* comprises a large part of the diet of the wild maned wolf. Of particular interest is the fact that this fruit is in the genus *Solanum*, which is widely known for the unique chemical constituents of many of its species. While some of these compounds are know for their therapeutic or medicinal uses, others have toxic qualities. Among the most widely studied categories of these chemical compounds are the glycoalkaloids, which are fairly common in ripe*Solanum* fruits, and can be toxic to vertebrates (Daroczy and Hernadi 1971, Cipollini and Levey 1997a, Wahaj et al. 1998). These secondary metabolites have been proposed to have numerous functions and effects, including chemical defense against pests and pathogens and alterations in gastrointestinal

motility which promote seed dispersion (Clench and Mathias 1992, Murray et al. 1994, Hopkins 1995, Cipollini and Levey 1997a, Cipollini and Levey 1997b, Wahaj et al. 1998). A considerable amount is known about the biological activity of glycoalkaloids from Solanum species used as important human foods, such as the tomato and potato (Roddick et al. 1990), however, very little comparable information exists on glycoalkaloids present in wild *Solanum* species such as *S. lycocarpum.* One report investigated the fruit of this plant in an attempt elucidate the chemical constituents and physiological mechanism responsible for its widespread use in the Brazilian cerrado for management of diabetes, obesity, and hypercholesterolemia (Dall'Agnol and Poser 2000). Phytochemical analysis revealed high levels of glycoalkaloids, including solasodine. These compounds were not detected in the medicinal preparation, however, and their effects on lowering blood glucose and cholesterol were attributed to high levels of polysaccharides (pectin, mucilage, starch). *Solanum lycocarpum* is also speculated to have therapeutic effects in the maned wolf that aid in protecting it from the renal parasite *Dioctophyma renale* (Langguth 1975).

It would be truly intriguing and even astounding if cystinuria was determined to be a component of the normal physiology in the maned wolf. The excretion of cystine and other amino acids in excess in comparison to other species would imply an evolutionary adaptation that serves a purpose in this species. Possible reasons for excretion of high levels of a substance in the urine under normal conditions would be to rid the body of a substance or metabolite that is accumulating in the body, to bond to or neutralize other substances in the urine, or to produce a scent or chemical signal

used in the animal's social structure. One physiologic peculiarity of the maned wolf (both captive and wild) which is readily noticeable, is the unique and strong odor of their urine and feces. The volatile compounds responsible for the odor are yet unidentified, as is their role in the natural history of the maned wolf. Urine and feces are commonly used as chemical signals in the animal kingdom. Roles of olfactory messages produced by animals include scent marking to define territorial ranges (Ralls 1971, Gosling 1982), to elicit sexual and maternal behavior, as gender and reproductive stage indication, as a defense mechanism, and many others (Eisenberg and Kleiman 1972, Doty 1986, Halpin 1986).

Chemical communication in the *Canidae* has been reported to be quite complex, although the complexity increases with increasing social complexity. For example, the gray wolf (*Canis lupus*), which is a pack animal, has a more complex social and signaling structure than does the more solitary red fox (*Vulpes vulpes*). Chemo-olfactory communication in both of these species has been studied in some depth, and urine and fecal marking for several purposes and in response to multiple cues has been demonstrated (Kleiman 1966, Peters and Mech 1975, Henry 1977, Jorgenson et al. 1978, Henry 1979, Bailey et al. 1980, Wilson et al. 1980, Whitten et al. 1980, Raymer et al. 1984, Raymer et al. 1986, Asa et al. 1990).

Based on what is known about the natural behavior of the maned wolf in the wild, it is assumed that their urine and feces does play an important role in their social and behavioral structure. For example, maned wolves habitually deposit their odiferous feces in elevated and prominent positions along the borders of their territories in the wild (Dietz 1984, Dietz 1987). Urine is also deposited along

territorial boundaries, and is occasionally used to mark food items and scats (Kleiman 1972, Dietz 1984). A defense or alarm function for defecation has also been proposed, as when startled or confronted from a close proximity, the maned wolf will often defecate prior to fleeing (Dietz 1984).

Chemical signals are used in abundance by animals, and even by some plants. They serve to trigger a response in a receiving organism, and in this context, are considered to be pheromones (Eisenberg and Kleiman 1972). There is a tremendous variety of chemical signals, many of which are very complex. In most cases, these chemicals are by-products of the organism's metabolism. Therefore, factors affecting the chemical composition of these substances might include genetic control mechanisms, environmental factors, hormonal signals, and dietary constituents.

Sulfur-containing compounds are commonly used as chemo-olfactory messengers in the animal kingdom, including among canids and felids (Jorgenson et al. 1978, Bailey et al. 1980, Wilson et al. 1980, Raymer et al. 1984, Raymer et al. 1986, Brahmachary and Dutta 1987). For example, felinine, a sulfur-containing amino acid, is found in the urine of members of the Felidae family. This amino acid is a highly regulated metabolite used in scent marking (Roberts 1963, Hendriks et al. 1995a, Hendriks et al. 1995b, Tarttelin et al. 1998). Therefore, the possibility that the compound(s) responsible for the strong odor of maned wolf urine and feces is a thiol compound, as well as the possibility that the metabolism of this compound is related to the metabolism of cysteine, cystine, or methionine, should be considered.
It is assumed by most that the molecular basis and pathogenesis of cystinuria in the maned wolf parallels that found in humans and dogs, and although this has not yet

been scientifically proven, the probability that cystinuria in maned wolves has a genetic origin is high. The presence of a renal tubular defect in cystine reabsorption that is genetically based and present in the majority of both captive and wild maned wolves could be disastrous to the future of this species. Successful management of such an obstacle would require determination of the prevalence of the disorder in wild populations, identification and characterization of the molecular and genetic basis of the disorder, the development of a diagnostic test to determine its presence, the identification of carrier animals, and the development of a successful breeding and reintroduction program that does not include affected animals. In the meantime, a nationwide or worldwide program should be structured to identify descendents of animals which have had cystine urolithiasis, perform routine checks for crystalluria on all wolves, and perform routine cyanide-nitroprusside tests. It is very likely that animals with strong-positive reactions on the cyanide-nitroprusside test as well as exhibiting cystine crystalluria, are positive for cystinuria and should be eliminated from the captive breeding program. Until such preventative measures and investigations are performed, our efforts will focus on addressing the management of cystinuria and prevention of clinical disease in captive populations of the maned wolf through nutritional modification.

1.3.6 *Previous Research on Nutritional Management of Cystinuria in the Maned Wolf*

Previous research on the nutritional management of cystinuria in captive maned wolves resulted in dietary modifications being made with the main goal of

better approximating the composition of the wild diet (Boniface 1998). This diet, as compared to a typical domestic canine maintenance formula, had higher levels of fat and moderate levels of protein. The fiber levels were increased through the addition of primarily soluble fiber (e.g. tomato pomace) in an effort to improve stool quality, which was commonly very poor, and even watery, in captive maned wolves. Also, the level of dietary sodium was increased to promote increased water consumption with the thought that this would in turn increase cystine solubility through dilution. In addition, based on the previously discussed research performed in humans and dogs, the dietary content of cysteine and methionine was decreased.

The wolves maintained on this diet exhibited markedly improved stool quality, presumably due to the high fiber levels. Moreover, the reduced cysteine and methionine levels in this diet were demonstrated to result in significantly lower urinary cystine concentrations in the maned wolves studied (Boniface 1998). Only a mild increase in average urine pH was seen, and this was not statistically significant. This diet formula was manufactured commercially, and beginning in 1998, was fed to nearly all maned wolves in the United States. Following implementation of this diet change, the incidence of clinical disease related to cystinuria was reportedly significantly decreased, however no widespread surveys or studies have been done to confirm this (M. Rodden, personal communication, 2000).

1.3.7 *Potential Effects of a Therapeutic Diet on Nutrient Availability*

A therapeutic diet has specifically designed nutrient formulations that have a targeted impact on the health of the animal consuming the diet. They are intended to

aid in the recovery from, or progression of, a particular disease condition. Therefore, because the commercially manufactured maned wolf maintenance diet is intended for the prevention of clinical signs related to cystinuria, this diet can be classified as a therapeutic diet. As with any therapeutic diet, certain nutrients are either reduced or elevated above typical levels in order to achieve the desired effect. In the current maned wolf diet based on the above research, for example, there are reduced levels of sulfur amino acids and increased levels of fiber and sodium.

There are several important considerations when implementing a therapeutic diet. First of all, it is imperative to determine if the diet will be suitable for long-term use in a particular patient, or if its desired effect can only be safely achieved with short-term limited use as needed to assist in controlling a disease process. Furthermore, it is important to know if the implemented changes in particular nutrients have any interacting effects on other nutrients in the diet. Finally, in domestic animal nutrition, therapeutic diets are formulated with changes deviating from what is already known about the maintenance nutrient requirements for a particular species. In the case of the maned wolf, requirements are not known for any macro- or micronutrients. Therefore, the challenges of formulating a balanced and safe yet effective therapeutic diet are compounded by a lack of any knowledge about the basic nutritional needs of this species.

For example, the high level of soluble fiber in the commercial maned wolf diet has the potential for numerous effects on the availability of nutrients. Probably the most prominent effect of fiber is on the digestibility of a diet, with increasing levels resulting in decreased digestibility (Burrows et al. 1982, McDonald et al.

1995). The digestibility of the diet may also be indirectly altered by fiber's effects on the rate of passage of digesta through the gastrointestinal tract of the wolves, since, depending on the type of fiber and how it is processed, fiber may either reduce or lengthen transit time (Smith and Eastwood 1980, Burrows et al. 1982). Rate of passage can affect digestibility through changes in the length of time of exposure of the feed to digestive enzymes and absorptive surfaces, as well as through changes in the intestinal microbial flora and alterations in food consumption ability (Maner et al. 1962, Kass et al. 1980, Krogdahl 1986).

Another example of a potential effect of the nutrient alterations in this therapeutic maned wolf diet on the overall health and nutritional status of the wolves concerns the low cysteine and methionine levels. Although several sources advocate this approach for the management of cystinuria in humans and dogs, and cysteine deficiency through excess renal loss is supposed to be prevented by adequate intestinal absorption of cystine, it has been suggested in the human literature that cystinuric patients maintained on low sulfur amino acid diets may develop sulfur amino acid deficiencies (Hellier et al. 1970, Silk 1974, Rodman et al. 1984, Osborne et al. 1989a, Martensson et al. 1990). Furthermore, the potential consequences of marginal to deficient cysteine status in relation to other nutrients, such as taurine, must also be considered. Taurine, which in domestic canids is synthesized in the liver from cysteine, has major functions in nearly all body systems including the liver, retina, heart, immune system, and coagulation cascade (Hickman et al. 1992, Chapman et al. 1993, Stapleton et al. 1998, Miglis et al. 2002, Militante and Lombardini 2002, Schuller-Levis and Park 2003). Despite the hepatic biosynthetic

capability in canids, however, taurine deficiency has been reported in this animal group (Moise et al. 1991, Kittleson et al. 1997, Backus et al. 2003, Fascetti et al. 2003). This may be in part due to the obligation of dogs to conjugate bile acids with taurine only. Therefore, situations resulting in increased bile acid turnover or loss may predispose dogs to taurine deficiency. Taurine deficiency has been reported with the most frequency in felids, in which taurine is considered an essential amino acid due to their reduced or absent ability to synthesize taurine in the liver (Knopf et al. 1978, Hayes and Trautwein 1989, Sturman 1992). Clinical signs of taurine deficiency are well documented in felids and include most commonly dilated cardiomyopathy, central retinal degeneration, and poor reproductive performance (Hayes et al. 1975, Barnett and Burger 1980, Sturman 1986, Pion et al. 1987, Dieter et al. 1993).

1.4 Hypotheses

1 An increase in the plant-based protein in the commercially available maned wolf maintenance diet will result in an increase in average urine pH, thereby increasing the solubility of cystine. Nutrient digestibility of such a diet change would need to be explored.

2 A decrease in the sodium content of the commercially available maned wolf maintenance diet will result in a decrease in urinary cystine levels.

3 The unique and strong odor of the urine and feces of the maned wolf may be due to a sulfur-containing compound.

4 The maned wolf has a rapid rate of passage of digesta as compared to the domestic dog and thus direct application of domestic dog nutrient requirements may not be advisable.

1.5 Main Objectives

The primary objective of this project was to reduce the incidence of clinical disease secondary to cystinuria in captive maned wolves without the use of pharmacologic agents. By making further modifications to the commercially available maintenance diet that is already in widespread use, we hoped to achieve a significant reduction not only in cystine excretion, but also an increase in cystine solubility in the urine. Moreover, through these studies, we aimed to increase the knowledge about the general physiology of the maned wolf and how these findings might affect our ability to deal with cystinuria in this species. And finally, an assessment of the current diet, as well as any new diets developed during the course of these studies, on the overall long-term health of the maned wolves, is imperative to ensure that this therapeutic diet not only accomplishes our goal of decreasing clinical disease from cystinuria, but is also safe and nutritionally balanced for this species.

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CHAPTER 2: Effects of Dietary Protein Source and Dietary Sodium to Potassium Ratio on Urine pH and Urine Cysteine Excretion in the Maned Wolf, *Chrysocyon brachyurus*

2.1 Abstract

This study was performed to assess the effect of dietary protein source on urine pH as well as the effect of dietary sodium level on urine cystine excretion in captive maned wolves. Four maned wolves were tested for two weeks on four experimental diets in a Latin square design. The four diets were the result of a two by two factorial arrangement of plant protein level (moderate or high level of plant-based protein) and sodium to potassium ratio (0.83 vs. 0.25). For each wolf, weekly measures of urine pH and assessments of urine cystine excretion (prevalence of urine cystine crystals, a cyanide-nitroprusside test, and urine cysteine levels) were performed. The high plant-based protein diets resulted in an increase ($P<0.05$) in average urine pH from 6.34 (moderate plant-based protein diets), to 6.65. There was also a significant effect of plant-based protein level on urine cystine crystal formation and the cyanide-nitroprusside test ($P<0.05$), with the moderate plant-based protein diets resulting in higher crystal prevalence and an increased score on the cyanide-nitroprusside test. Changes in the dietary sodium to potassium ratio did not effect ($P>0.05$) urine cystine excretion. These results suggest that, independent of sulfur amino acid content, plant-based protein levels (high or moderate plant-based protein) can have a significant effect on urine pH and urinary cysteine excretion in maned wolves.

2.2 Introduction

The maned wolf, *Chrysocyon brachyurus*, is a rare South American canid of which there are currently less than 100 maintained in zoological parks across the United States (M. Rodden, personal communication, 2001). The most serious and widely recognized medical condition afflicting captive maned wolves is cystinuria (Bovee and Bush 1978, Bush and Bovee 1978, Bovee et al. 1981, Mussart and Coppo, 1999). Cystinuria has been well characterized in both humans and domestic dogs and in these species has been determined to be an autosomal recessive metabolic abnormality resulting in the defective intestinal absorption and renal reabsorption of cysteine and the dibasic amino acids lysine, ornithine, and arginine (Harris et al. 1955, Rosenberg et al. 1965, Casal et al, 1995). Clinical manifestations of cystinuria develop only secondary to the formation of cystine uroliths which can cause life-threatening upper or lower urinary tract obstruction, as well as predispose the animal to complications such as urinary tract infections and renal insufficiency (Lindell et al. 1997, Rutchik and Resnick 1997, Joly et al. 1999, Goodyer et al. 2000). Maned wolves in captivity have historically been plagued by complications related to cystinuria, such as cystic and renal calculi, which result in significant morbidity and mortality (Bovee and Bush 1978, Bush and Bovee 1978, Bovee et al. 1981, Mussart and Coppo 1999).

Medical management and prevention of clinical disease in both human and canine cystinuria revolve around the following two main goals: increasing the urinary solubility of cystine and reducing urine cystine concentration. These objectives are typically accomplished through a combination of dietary modification, urine

alkalinization, and the administration of thiol-containing drugs (Joly et al. 1999, Ng and Streem 1999, Goodyer et al. 2000). Due to the high prevalence of cystinuria in captive maned wolves in the United States, and the high incidence of undesirable side effects of available pharmacologic agents in humans and domestic dogs including gastrointestinal upset, dermatologic conditions, hematologic abnormalities, and protein-losing nephropathy (Halperin et al. 1981, Joly et al. 1999, Barbey et al. 2000), dietary modification has been investigated as a method of reducing and preventing clinical disease from cystinuria in captive maned wolves.

Previous research in this area resulted in the production of a commercially manufactured maned wolf diet (Boniface 1998). This diet, which was formulated to contain low protein and low levels of sulfur-containing amino acids as compared to a typical canine maintenance formula, resulted in a significant reduction in urine cystine concentrations in the wolves studied. Reduction of dietary protein intake, especially that of animal origin, has been demonstrated to reduce urinary cystine excretion in humans and the domestic dog (Rodman et al. 1984, Osborne et al. 1989a). This effect is likely due to the decreased intake of methionine, a precursor of cysteine, which tends to be higher in animal-based proteins as compared to plant-based proteins (Kolb et al. 1967, Osborne et al. 1987).

Furthermore, an alteration in the type of dietary protein would likely also have an effect on urine cysteine solubility mediated by changes in urine pH. Of the four amino acids consistently excreted in excess in cystinuria, cysteine is the least soluble and crystallizes in supersaturated urine. The solubility of cysteine is highly dependent on pH, being relatively insoluble at normal urine pHs of 5 to 7 (Osborne et

al. 1989b). The solubility of cysteine increases with increasing urinary pH, almost doubling at pH 7.8 and tripling at pH 8.0 (Dent and Senior 1995). Herbivores have higher urine pH than carnivores. It has been hypothesized that the reason for the marked difference in urine pH between herbivores and carnivores stems from the lower sulfur amino acid content of plant versus animal-based proteins. This hypothesis did not hold true in a previous study done with maned wolves where a diet containing low levels of sulfur containing amino acids did not have a significant effect on urine pH (Boniface 1998).

In the commercially manufactured maned wolf diet developed based on the work described by Boniface (1998), sodium (Na) levels had been increased in an attempt to encourage increased water intake and therefore increase cysteine solubility through dilution. In this diet, potassium (K) levels were not changed when Na was increased and this had an impact on Na:K ratios as well as dietary electrolyte balance. Furthermore, this increase in Na may have been contraindicated, given that in human studies, urinary excretion of cysteine has been demonstrated to be directly correlated with urinary Na excretion, with low Na diets effectively reducing urinary cysteine excretion (Jaeger et al. 1986, Norman and Manette 1990, Peces et al. 1991, Rodriguez et al. 1995). Given the evidence from the literature on the strong relationship between herbivorous diets and high urine pH, the lack of effect of low sulfur-containing amino acid diets on urine pH in maned wolves, and the direct positive correlation between dietary Na level and cysteine excretion in humans, further studies attempting to increase urine pH and reduce urine cysteine excretion through dietary modifications appeared to be warranted.

The objectives of the present study were two-fold: 1) to evaluate the effect of animal vs. plant-based dietary protein, with a constant sulfur amino acid content, on the urine pH of the maned wolf and 2) to examine the effect of high vs. low dietary sodium levels, resulting in a high vs. low Na to K ratio, on urine cysteine excretion in the maned wolf.

2.3 Materials and Methods

2.3.1 *Animals*

Four captive born maned wolves were used in this study. Subjects included three intact females (ages 7, 10, and 11 years) and 1 intact male (age 9 years). All wolves were maintained at the National Zoological Park's Conservation and Research Center in Front Royal, Virginia, and the study adhered to this institution's animal care and use protocols. Wolves were housed separately, in their normal enclosures, throughout the trial. Enclosures included constant indoor (concrete room with den) and outdoor (large fenced-in yard) access. Details regarding den and enclosure parameters have been published (Brady and Ditton 1979). All wolves were determined to be clinically healthy, although one female wolf (age 11) was maintained on carprofen (50mg) orally twice daily for osteoarthritis.

2.3.2 *Diets*

A total of four dietary treatments were tested. To achieve the four dietary treatments, two isocaloric and isonitrogenous basal diets with different levels of plant

and animal protein sources were formulated to contain similar amino acid levels and were manufactured by Purina Mills Inc. (Table 2-1). These diets were analyzed for amino acids, minerals, and fiber. The two basal diets were extruded into a medium-size kibble to mimic processing in the commercial maned wolf diet.

For experimental purposes, the two basal diets were ground (to pass through a 0.623 mm screen) and then mixed with the appropriate amounts of sodium chloride and potassium chloride to achieve the desired Na:K ratios (Table 2-2) and generate four (A, B, C, and D) experimental diets. Diets A and B were made from the moderate plant-based protein basal diet, while diets C and D were made from the high plant-based protein basal diet. Diet A was the control diet, formulated based on the commercial maned wolf diet developed from the Boniface (1998) study and which the wolves had already been maintained on for approximately two years prior to the start of this trial. Diet B was the same basal diet as Diet A (moderate plant-based protein basal diet) but had a lower Na to K ratio (Table 2-2). Diets C and D were formulated to be identical in nutrient content as diets A and B, respectively, except that the ingredient protein sources were primarily of plant origin. The animal-derived protein sources were primarily meat meal and poultry meat meal (Table 2-3). The plant-derived protein sources were primarily soybean meal, corn gluten meal, and soy protein isolate.

Table 2-1: Formulated ingredient and nutrient composition of the basal diets.

Ingredient	Plant-based Protein	
	Moderate	High
	% as fed	
Meat Meal	9.58	0.00
Low Ash Poultry Meat Meal	2.00	0.00
Dried Whey	0.50	0.50
Brewer's Yeast	2.00	2.00
Beef Digest[1]	3.00	3.00
Dehulled Soybean Meal	11.81	21.50
Corn Gluten Meal	0.00	3.45
Soy Protein Concentrate	0.00	0.20
Rice Flour	25.00	25.00
Corn Flour	9.35	0.00
Poultry Fat	6.00	6.00
Bleachable Fancy Tallow	6.88	8.47
Soy Oil	0.50	0.50
Ground Beet Pulp	4.00	11.21
Apple Pomace	7.00	7.00
Ground Soy Hulls	6.44	2.77
Tomato Pomace	2.50	2.50
Sodium Chloride	0.35	0.39
Lysine	0.00	0.02
Other	3.09[2]	5.49[3]
Formulated Nutrient Levels (analyzed levels in parentheses)		
Protein, %	18.50 (18.81)	18.50 (18.55)
Fat, %	16.00 (17.2)	16.00 (16.9)
Crude fiber, %	6.5	6.5
Neutral detergent fiber, %	13.77	15.69
Acid detergent fiber, %	9.38	10.01
Insoluble fiber, %	(14.8)	(16.9)
Soluble fiber, %	(3.25)	(4.32)
Metabolizable Energy, kcal/kg	3530	3530
Methionine, %	0.26 (0.51)	0.27 (0.46)
Cystine, %	0.21 (0.37)	0.22 (0.39)
Lysine, %	0.95 (1.04)	0.98 (1.05)
Sodium, %	0.20	0.20
Potassium, %	0.60	0.60

[1] A palatability enhancing liquid ingredient with less than 10% dry matter.

[2] Contains (percentage as fed): dicalcium phosphate 1.053, calcium carbonate 0.282, pyridoxine (1%) 0.144, choline chloride (70%) 0.141, menadione (2900 ppm) 0.103, vitamin D_3 (7500 IU/g) 0.053, biotin (0.1%) 0.050, vitamin E (500 IU/g) 0.45, vitamin A (27240 IU/g) 0.037, ferrous sulfate (31%) 0.030, calcium iodate (60%) 0.025, zinc oxide (72%) 0.022, L-lysine 0.022, ethoxyquin 0.018, selenium (0.06%) 0.015, calcium pantothenate (17.6 g/kg) 0.013, folic acid (2%) 0.013, thiamin (10%) 0.012.

[3] Contains (percentage as fed): dicalcium phosphate 2.713, calcium carbonate 0.172, pyridoxine (1%) 0.142, choline chloride (70%) 0.145, menadione (2900 ppm) 0.103, vitamin D_3 (7500 IU/g) 0.53, biotin (0.1%) 0.05, vitamin E (500 IU/g) 0.045, vitamin A (27240 IU/g) 0.037, ferrous sulfate (31%) 0.030, calcium iodate (60%) 0.025, zinc oxide (72%) 0.022, ethoxyquin 0.018, selenium (0.06%) 0.015, calcium pantothenate (17.6 g/kg) 0.013, folic acid (2%) 0.013, thiamin (10%) 0.012.

Table 2-2: Protein sources and sodium and potassium levels in the experimental diets, as formulated.

Diet	Animal Protein, %[1]	Plant Protein, %[1]	Na, %	K, %	Na:K
A	43.8	56.2	0.5	0.6	0.83
B	43.8	56.2	0.2	0.8	0.25
C	5.3	94.7	0.5	0.6	0.83
D	5.3	94.7	0.2	0.8	0.25

[1]Percent of the total protein in the diet composed by either animal or plant protein.

Table 2-3: Ingredient contributions to protein content of experimental diets

	Moderate Plant-based Protein Basal Diet			High Plant-based Protein Basal Diet		
Ingredients	***% of Diet***	***% Protein in Ingredient***	***% of Total Protein [1]***	***% of Diet***	***% Protein in Ingredient***	***% of Total Protein [1]***
Rice Flour	25.00	7.1	9.60	25.00	7.1	9.70
Soybean meal	11.80	50.1	32.10	21.50	50.1	58.70
Meat meal	9.58	59	30.80	0.00	59	0.00
Corn Flour	9.35	7.8	3.94	0.00	7.8	0.00
Apple Pomace	7.00	4.4	1.63	7.00	4.4	1.69
Soy Hulls	6.44	10.5	3.60	2.77	10.5	1.58
Beet Pulp	4.00	11.2	2.40	11.21	11.2	6.87
Corn Gluten Meal	0.00	65.5	0.00	3.45	65.5	12.32
Tomato pomace	2.50	21.6	2.93	2.50	21.6	2.94
Brewer's yeast	2.00	45.9	5.10	2.00	45.9	5.01
Low ash PMM[2]	2.00	68	7.50	0.00	68	0.00
Dried whey	0.50	12.1	0.40	0.50	12.1	0.32
Soy protein concentrate	0.00	80	0.00	0.20	80	0.87
Total % of diet	**80.16**			**76.13**		
% Animal-based protein [3]			43.80			5.33
% Plant-based protein [3]			56.20			94.67
Protein in diet from above ingredients, %			**18.38**			**18.34**

[1] % of total protein in the diet that is derived from animal sources or plant sources

[2] PMM = poultry meat meal

3 % of the diet that this ingredient contributes to the total protein in the diet

2.3.3 *Experimental Design*

The experiment was designed as a 4x4 Latin square. Each maned wolf participating in the experiment was tested once on each of the four diets. The diet fed for each two-week period was changed, such that all wolves consumed each of the diets at some point during the four experimental phases. All diets were represented at least once in each of the experimental phases.

2.3.4 *Diet Trial*

Each of the four wolves was randomly assigned to one of the four experimental diets within each phase. A phase was defined as a 14-day period over which the first four days were an adaptation period to the new diet. The adaptation period was included to prevent any decreases in consumption or rejection of the new diet. To ensure a smooth transition, a slow incorporation of the new diet was done as follows: the diet on which the wolves were maintained in the previous phase was mixed in decreasing ratios with the new diet during the first 4 days of each phase in ratios of previous diet to new diet of 3:1, 1:1, 1:1, and 1:3. Fresh diet was offered daily at which time food disappearance (food consumption) for the previous day was determined.

2.3.4.1 **Urine pH**

A voided urine sample was collected at days 7 and 14 in each two-week phase, and urine pH was measured using an electronic pH meter immediately after collection. Urine was collected in different ways depending on the typical behavior of

each individual wolf, with methods including collection of fresh urine from the floor in dens, and collection of urine voided onto plastic or metal objects placed in enclosures to encourage territorial marking. All urine samples were collected first thing in the morning and prior to the wolves being fed, in order to avoid a postprandial “alkaline tide” (Niv and Fraser 2002) effect on urine pH.

2.3.4.2 Urine Cysteine Levels

On each voided urine sample (days 7 and 14 of each phase), microscopic analysis was performed to determine the presence and prevalence of cystine crystals. The number of cystine crystals per field (10x) was graded on a scale of 1 to 4 with grade 1: no crystals, grade 2: 5-10 crystals, grade 3: 5-20 crystals, and grade 4: greater than 20 crystals. Urine samples negative for cystine crystals (grade 1) were refrigerated for 3 days and re-evaluated for presence and prevalence of crystals. Samples still negative for cystine crystals post-refrigeration that had a pH>7.0 were acidified to a pH just below 7.0 with acetic acid and evaluated immediately for cystine crystals. None of the samples originally graded 1 demonstrated a change in grade following refrigeration and acidification (data not shown).

A sodium cyanide nitroprusside test (Eagle Nitroprusside Test Procedure, Cima Scientific™, Dallas, TX) was performed on urine samples collected on day 14 of each phase. Two drops of urine were added to a clean glass test tube. One drop of cyanide reagent (sodium cyanide solution 67g/l) (*ReagentPlus*,™ Sigma-Aldrich Co., St. Louis, MO) was added to the urine and mixed well. The mixture was allowed to sit undisturbed for 10 minutes. One drop of nitroprusside reagent (sodium

nitroprusside solution 50g/l) (*ReagentPlus*,™ Aldrich Chemical Co., St. Louis, MO) was added, and the sample was observed for a color change. An immediate pink to purple color change indicates the presence of cystine or homocystine. Color intensity, which is dependent on cystine concentration in the urine sample, was subjectively graded on a scale of one to four, by the same person each time, as negative (1), weak positive (2), positive (3), or strong positive (4).

Urine samples collected from day 14 of each two-week phase were analyzed for complete amino acid profiles, including cysteine, ornithine, lysine, and arginine. Samples were analyzed at the Amino Acid Analysis Laboratory, Department of Molecular Biosciences, University of California at Davis. Within 20 minutes after collection, samples were frozen at -20ºC, and within 8 hours were frozen at -80ºC. Amino acid concentrations were determined by the use of an automated analyzer (Model 7300, Beckman Instruments, Palo Alto, CA), which utilizes cation-exchange chromatography and spectroscopic determination of a ninhydrin reaction with amino acids to obtain measured values from a urine sample. Norleucine was used as an internal standard to standardize the concentrations of amino acids across time using different batches of reagents. For determination of sulfur-containing amino acids, a portion of the urine sample was deproteinized with an equal volume of 6% sulfosalicylic acid, centrifuged at 14,000 rpm for 25 minutes, and the supernatant analyzed using the automated analyzer as described above. Each urine sample was also analyzed for creatinine, so that amino acid levels could be expressed in relation to creatinine. Creatinine analysis was performed by the Iowa State University College of Veterinary Medicine Clinical Pathology Laboratory using the Hitachi 912 Clinical

Chemistry Analyzer. This analyzer employs the picric acid method of creatinine analysis followed by spectrophotometric assay.

2.3.5 *Statistical Analysis*

The data were analyzed using a double blocking approach with animal and phase (time period) as random effects. Within each phase, the experiment was a factorial arrangement of two plant-based protein levels and two sodium to potassium ratios. Multivariate analysis was used to determine the relationship between the variables tested. The JMP statistical program of SAS (2000) was used to perform the analysis. Significance was accepted at $P<0.05$.

2.4 Results

There were no interactions between plant-based protein level and Na to K ratios on any of the parameters measured, thus results and discussion will focus on main effects. Analysis of daily food consumption levels demonstrated that there were no differences ($P>0.05$) in consumption between the diets tested (data not shown).

2.4.1 *Urine pH*

Feeding the diets with the high plant-based protein level (diets C and D) resulted in a higher ($P<0.05$) average urine pH than when the diets with the moderate plant-based protein level (diets A and B) were fed with main effect averages of 6.65 and 6.34, respectively (Table 2-4). This resulted in a significant effect ($P<0.05$) of

plant-based protein level on the urine pH of the wolves. There was no detectable effect of electrolyte balance on urine pH.

2.4.2 *Urine Cysteine Levels*

2.4.2.1 Microscopic Analysis

Diet plant-based protein level but not Na to K ratio had an effect ($P<0.05$) on the presence and prevalence of cystine crystals, with wolves fed the moderate plant-based protein-based diets having a higher incidence of cystine crystal formation (Table 2-4).

2.4.2.2 Cyanide Nitroprusside (CN) Test

As observed with both urine pH and urine cystine crystal prevalence, diet plant-based protein level but not Na to K ratio had an effect ($P<0.05$) on the CN test. Feeding the high plant-based protein diet resulted in lower ($P<0.05$) CN test results than when the moderate plant-based protein diets were fed (Table 2-4).

2.4.2.3 Urine Amino Acid Levels

Urine samples from day 14 of each phase were analyzed for urine cysteine levels (Table 2-4). No significant effect of diet ($P>0.05$) was observed on urine cysteine levels. There was, however, a significant random effect of animal ($P=0.005$, data not shown).

Table 2-4: Impact of plant-based protein level and sodium to potassium ratio on urine pH, cyanide-nitroprusside (CN) test and urine cystine crystal formation.

Diet	Plant Protein level	Sodium (Na) (%)	Potassium (K) (%)	Na:K Ratio	Average Urine pH	CN[1] Test	Cystine Crystals	Urine Cysteine[3]
A	Moderate	0.5	0.6	0.83	6.30	2.68	1.75	1347
B	Moderate	0.2	0.8	0.25	6.39	2.56	1.86	1429
C	High	0.5	0.6	0.83	6.65	2.81	1.70	1349
D	High	0.2	0.8	0.25	6.66	2.44	1.70	1627
				SEM	0.0348	0.400	0.462	342
				P value	0.0039	0.021	0.0481	0.8255
				MAIN EFFECTS[2]				
				Plant protein				
				Moderate	6.34	3.00	2.13	1388
				High	6.65	2.25	1.38	1488
				P value	0.0006	0.014	0.0479	0.7668
				Electrolyte				
				High Na:K	6.47	2.62	2.00	1348
				Low Na:K	6.52	2.62	1.50	1528
				P value	0.446	1.00	0.1546	0.5947

[1] Cyanide nitroprusside test.
[2] There was no interaction between plant-based protein level and Na to K ratio.
[3] nmol/mg creatinine

2.5 Discussion

Daily food consumption was closely monitored during this trial primarily because of palatability concerns associated with the low Na levels and changes in diet ingredients from animal-based to plant-based. In humans, although low dietary Na has been directly correlated with decreased urinary cystine excretion, compliance is typically poor due to the decreased palatability of low sodium diets (Jaeger et al. 1986, Peces et al. 1991, Rodriguez et al. 1995). In this trial, there was no difference in consumption of any of the four diets, indicating that palatability of the low vs. high Na diets or the changes in ingredients in the moderate and high plant-based protein diets were not an issue in these maned wolves.

The kidneys play a major role in adjusting hydrogen ion concentrations of the extracellular fluid by excreting acidic or alkaline compounds at varying rates. Nonvolatile acids, produced mainly by the metabolism of proteins, are removed from the body via renal excretion. Dietary contributions to the daily acid load are derived primarily from the intake of sulfur-containing amino acids such as cysteine and methionine. Animal proteins, due to their higher levels of sulfur-containing amino acids, are more acidifying than plant proteins when consumed (Halperin and Jungas 1983, Swenson 1993, Burkholder 2000), and therefore, carnivores typically have low urine pHs while herbivores excrete a more alkaline urine. For example, the average urinary pH of the domestic cat, a true carnivore, is between 6.1 and 6.4 (Allen et al. 1997), while that of a domestic ruminant is typically greater than 7.4 (Divers and VanMetre 1996). In this maned wolf study, the sulfur amino acid (cysteine and methionine) level of the basal diets was formulated to be the same despite differences

in the protein sources. Upon analysis, the moderate plant-based protein diet had a slightly higher sulfur amino acid content than the high plant-based protein diet, but this difference was within the amino acid analytical error. It can therefore be considered that there is some other chemical property of plant-based proteins, other than differences in sulfur amino acid content, that can affect urine pH in the maned wolf.

In the two diets used in this trial, sulfur amino acid levels (methionine and cysteine) were higher than formulated levels. This difference reflects nutrient variability in ingredients, as well as insufficient knowledge of exact amino acid levels in some of the ingredients used. The sulfur amino acid levels are consistent between the two diets, however, and thus conclusions reached above regarding urine pH in relation to sulfur amino acid level versus protein source are still valid.

The identification of flat, colorless, hexagonal crystals in a urine sediment is strongly suggestive of cystinuria. However, this finding is present only in a minority of both human and canine patients with cystinuria (Rutchik and Resnick, 1997, Osborne et al. 1999). In alkaline urine samples, crystal formation may be promoted by refrigeration and acidification of the urine to a pH <7.0 with hydrochloric or acetic acid (Osborne et al. 1995, Gregory and Schwartz 1998). In the cyanide-nitroprusside (CN) test, cyanide reduces the disulfide linkage in cystine and homocystine to the free sulfhydryl groups to produce a color change from light pink to purple, the intensity of which is dependent on urinary concentration of cystine or homocystine. The CN test is able to detect urinary cystine levels as low as 75 to 125 mg/g of creatinine (Rutchik and Resnick, 1997). The prevalence of cystine crystals as well as the results of the

CN test are relatively crude methods of monitoring urine cystine concentration, however, we found that these measures were sensitive enough to detect an effect of diet. The prevalence of urine cystine crystals and the cyanide nitroprusside test were also useful in a qualitative fashion, as two of the study animals were consistently positive on both tests throughout the trial, while two were not. These rapid, inexpensive, and easy to perform tests therefore could be useful screening tools to identify and monitor cystinuria-positive animals in a collection.

There was no effect of experimental diet on any single urine cysteine level. It is possible that the very strong effect of animal on the analyzed urine cystine levels could have masked any potential effect of diet. The mean urine cysteine concentration across the four diets was 2050, 1311, 692, and 1698 nmol/mg creatinine (standard error of 208, P=0.0071) for animals 1, 2, 3, and 4, respectively. When given in nmol per ml of urine, the mean urine cystine concentrations become 3879, 653, 1100, and 2269. The upper limit of cystine solubility in human urine is 2500nmol per ml of urine (300mg/L), however, there are numerous discrepancies between reported normal and abnormal urinary cystine levels in both humans and dogs (Hoppe 1994, Stoller et al. 1997). In one study, 7 out of 24 dogs with a history of cystine urolithiasis demonstrated urine cystine levels within the normal reference range reported for dogs (Hoppe et al. 1993). Therefore, the finding of a single urinary cystine value within the reported normal range on a single sample does not exclude the possibility of cystinuria in that patient. Suspicions of cystinuria should ideally be confirmed by urine cystine quantification over a 24-hour period (Rutchik and Resnick 1997, Ng and Streem 1999, Osborne et al. 1999), a test which is not feasible in maned wolves.

In conclusion, we found that a diet with the majority of its protein from plant sources results in a higher average urine pH. A higher urine pH should result in an increase in cystine solubility and therefore reduce the risk of cystine precipitation and urolith formation. The CN test and the prevalence of urine cystine crystals, which were used as crude measures of urine cystine concentration, are sensitive enough to demonstrate an effect of diet. In the maned wolf a high plant-based protein diet resulted in lower urine cystine crystal formation and lower positive results on the CN test. Both of these tests may be useful screening tests for maned wolves to identify cystinuria-positive animals. Single urine cysteine levels are subject to significant variability (Hoppe et al. 1993) and have been demonstrated to be an unreliable method of cystinuria detection and therapeutic monitoring in humans and domestic dogs. In this study, no effect was seen of dietary therapy on single urine cysteine levels. Unfortunately, more sensitive methods, such as 24-hour urine cysteine quantification, are not feasible in maned wolves.

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CHAPTER 3: Identification of Volatile Compounds in the Urine and Feces of the Maned Wolf, *Chrysocyon brachyurus*

3.1 Abstract

This study was performed to determine if sulfur-based chemical compounds could be identified in urine and feces of maned wolves that may be related to the high prevalence of cystinuria in this species, as well as be responsible for the unique and strong odor of maned wolf excreta. Fecal samples were collected from 5 maned wolves, 4 domestic dogs, and one clouded leopard, urine samples were collected from 7 maned wolves, 4 domestic dogs, and nine Mexican gray wolves, and enclosure air samples, using a solid phase microextraction (SPME) fiber, were collected from 5 maned wolves, 2 black-footed ferrets, 4 clouded leopards, and 3 Mexican gray wolves. All samples were analyzed using gas chromatography-mass spectrometry. Volatile constituents of the excreta and air samples overlapped significantly among all of the species tested. A high concentration of 2,5-dimethylpyrazine was detected in all urine samples collected from maned wolves, and was not identified in samples from any of the other species. A sulfur-based compound unique to maned wolf urine or feces was not detected. The potential roles of pyrazine compounds in the chemical communication of the maned wolf are discussed.

3.2 Introduction

The maned wolf, *Chrysocyon brachyurus*, is a rare canid species that inhabits the grasslands, swamplands, and scrub forests of central and eastern Brazil, northern Argentina, Paraguay, eastern Bolivia, and southeastern Peru (Langguth 1975, Dietz

1984). Habitat destruction is a major cause of this wolf's classification as "lower-risk, near-threatened" on the 2000 IUCN Red List, as endangered by the U.S. Fish and Wildlife Service, and its listing under Appendix II of the Convention on International Trade in Endangered Species (CITES). There is a relatively small captive population maintained in zoological parks around the world, with those in the United States managed under a Species Survival Plan (M. Rodden, personal communication, 2001). Many features distinguish the maned wolf from its canid relatives, justifying its classification in a separate genus, *Chrysocyon*, of which it is the sole member. For instance, the diploid chromosome number of *Chrysocyon* (78) differs from that of *Canis* (76) (Newnham and Davidson 1966, Wayne et al. 1987). Striking physical characteristics such as long slender legs and large erect pinnae are likely anatomical adaptations to facilitate locomotion and capture of prey in the tall grasses of their natural habitat (Kleiman 1972, Dietz 1984). One readily noticeable physiologic peculiarity of the maned wolf, both in the wild and in captivity, is the unique and strong odor of their urine and feces. The volatile compounds responsible for this odor have not previously been investigated.

The odor of urine plays a central role in the chemical communication of numerous animal species, and it is the volatile substances which vaporize from the urine surface that are typically detected as a "message". Some animal species manufacture original compounds for use as urinary pheromones. For example, felinine (2-amino-7-hydroxy-5,5-dimethyl-4-thiaheptanoic acid) is a sulfur-containing amino acid synthesized by, and excreted in, the urine of several members of the Felidae family (Westall 1953). This compound, which degrades to produce the

characteristic tom-cat urine odor, is thought to play a major role in territorial marking in numerous felid species (Hendriks et al. 1995a). In addition, the production of felinine, as well as being influenced by reproductive hormone levels, is strongly influenced by dietary factors (Datta and Harris 1953, Shapiro 1962, Hendriks et al. 1995b, Tarttelin et al. 1998). This is consistent with other examples demonstrating that diet can have a significant effect on urine composition and odor, such as the excretion of various sulfur-containing compounds in humans following consumption of asparagus (Gearhart et al. 1977, Waring et al. 1987). Physiological factors can also alter urine composition precipitating changes in odor. For example, numerous pathological metabolic abnormalities which generate a characteristic urinary odor have been identified in humans, such as maple syrup urine disease and trimethylaminuria (Najarian 1980, Burke et al. 1983, Podebrad et al. 1999, Mitchell and Smith 2001).

Cystinuria, a metabolic defect in renal and intestinal amino acid transport of cysteine and other dibasic amino acids, is an important and widely recognized condition afflicting captive maned wolves with a high prevalence (Bovee and Bush 1978, Bush and Bovee 1978, Bovee et al. 1981, Mussart and Coppo 1999). Cystinuria also occurs, with a much lower prevalence, in humans and domestic dogs, and has been demonstrated in these species to have a genetic basis (Rosenberg et al. 1965, Tsan et al. 1972, Palacin et al. 2000). The possibility that these amino acids or their breakdown products are responsible for the unique maned wolf urine odor must be considered, although such an odor has not been reported in humans or domestic dogs with cystinuria. The present study was conducted in an effort to determine the

class of compounds responsible for the unique odor of the urine of the maned wolf and assess a possible role or link of this compound to cystinuria, a metabolic disorder with a high prevalence in captive maned wolf populations.

3.3 Materials and Methods

3.3.1 *Fecal Samples*

3.3.1.1 Collection

Fecal samples less than 2 hours old were collected from animal enclosures. In animals with access to outdoor enclosures, extraneous debris (such as grass, dirt, etc.) was manually removed from the outer surface of the fecal mass prior to sample processing.

3.3.1.2 Species

Fecal samples were collected from five maned wolves, four domestic dogs (three Labrador retrievers and one Golden retriever) and one sample was collected from a clouded leopard.

3.3.1.3 Sample Processing

All fecal samples were processed within 4 hours of collection. Fifty grams of each fecal sample were mixed with 50 milliliters of cold double-distilled water. The sample was thoroughly mixed and then centrifuged for 10 minutes at 8,000 rpms. The resulting supernatant was collected and frozen (-20°C) until analysis.

3.3.2 *Urine Samples*

3.3.2.1 Collection

Fresh voided urine samples were collected by various methods depending on the individual animal and species. Methods used included holding animals in an indoor enclosure with a clean smooth floor off which urine could be collected, as well as taking advantage of behavioral characteristics including submissive responses and territorial marking.

3.3.2.2 Species

Urine samples were collected from seven maned wolves, four domestic dogs, and nine Mexican gray wolves.

3.3.2.3 Sample Processing

Urine samples (average volume 25-30mls) were collected and frozen at -20°C.

3.3.3 *Air Samples*

3.3.3.1 Collection

A Solid Phase Micro-Extraction (SPME) sampler (Supelco SPME Portable Field Sampler; 75um thick partially crosslinked carboxen/polydimethyldiloxane phase material, Supelco, Bellefonte, PA) was used to detect volatile odor components. The sampler was placed in the indoor enclosure of the animal, in close proximity to an actual urine or fecal sample, or in close proximity to a commonly

used marking site. The sampler was left in place for 20 minutes in a location where the animal could not access it.

3.3.3.2 Species

Samples were collected from five maned wolves, three Mexican gray wolves, two black-footed ferrets, and four clouded leopards.

3.3.3.3 Sample processing

Following use, the SPME samplers were kept refrigerated until analysis.

3.3.4 *Sample Analysis*

A standard swine odor solution was formulated based on the artificial slurry developed by Persaud et al. 1996. Additional odorous compounds that were consistently present in the initial air samples were identified and added to the standard solution. Standard solutions were prepared at concentrations of 100, 50, 25, 10, and 5% of the stock standard. The stock standard swine odor solutions were stirred for approximately 30 minutes prior to the removal of a 1ml sample that was placed in a 40ml glass vial with a Teflon-lined septum and hole cap. From the stock standards a linear prediction curve for individual odorant concentrations was generated using a Hewlett Packard 6890 Plus gas chromatograph, based on area under the chromatographic peak. The equation generated for each odorant was then used to predict unknown concentrations of odorants identified in the collected samples.

3.3.5 *Statistical Analysis*

Data were analyzed statistically when concentrations for at least one species were in the detectable range. Individuals within species were considered an experimental unit and thus there was unequal replication. Analysis of variance (Snedecor and Cochran 1989) to determine species effect was conducted using the MIXED procedure of SAS (Statistical Analysis System, 2000). When the overall model was significant ($P \leq 0.05$), pairwise comparisons using Tukey's HSD method (Tukey 1991) were performed. A probability of $P \leq 0.05$ was considered significant.

3.4 Results

Volatile chemical compounds identified in fecal samples of maned wolves, domestic dogs, and a clouded leopard are displayed in Table 3-1. Measurable concentrations (mM) identified in at least one animal included decane, nononal, phenol, indole, 2-methyl indole, 3-methyl indole, C2-acetic acid, C3-propionic acid, 2-methyl propionic acid, C4-butyric acid, 3-methyl butyric acid, pentanoic acid, undecane, dodecane, dimethyldisulfide, nonane, tridecane, and tetradecane. No significant differences were seen in fecal sample odor between maned wolves and domestic dogs. The clouded leopard sample had statistically higher concentrations of phenol ($P=0.0017$), 2-methyl propionic acid ($P<0.0001$), and tridecane ($P<0.0306$) than the maned wolves or domestic dogs but there was only an n of one for clouded leopard as a species and thus this would have to be replicated further before conclusions can be made.

Volatile compounds identified in urine samples of maned wolves, Mexican gray wolves, and domestic dogs are displayed in Table 3-2. Measurable concentrations (mM) identified in at least one animal included butyrolactone, decane, nononal, phenol, C2-acetic acid, C3-propionic acid, undecane, dodecane, nonane, 1-decane, tridecane, tetradecane, 2,5-dimethylpyrazine, 2-methyl-6-(1-propenyl) pyrazine, and trimethylpyrazine. The Mexican gray wolves had significantly higher concentrations of phenol ($P=0.0226$) than the maned wolves and domestic dogs, while the maned wolves had significantly higher concentrations of 2,5-dimethylpyrazine ($P<0.001$) than the Mexican gray wolves and domestic dogs.

Volatile compounds identified in air samples collected from the enclosures of maned wolves, Mexican gray wolves, clouded leopards, and black-footed ferrets are displayed in Table 3-3. Measurable concentrations (mM) identified in at least one animal included carbon disulfide, butyrolactone, decane, nononal, phenol, undecane, dodecane, nonane, 1-decane, tridecane, tetradecane, and 2,5-dimethylpyrazine. There were no statistically different concentrations of volatile compounds among species.

Table 3-1: Volatile chemical compound concentrations (mM) identified in feces collected from maned wolves (MW), domestic dogs (DD), and a clouded leopard (CL).

Chemical Compound (mM)[1]	Species			SEM[5]	P value
	MW[2]	DD[3]	CL[4]		
Decane	0.229	0.00	0.00	0.197	0.3215
Nononal	0.833	0.00	0.00	0.519	0.4586
Phenol	0.077[b]	0.005[b]	0.268[a]	0.065	0.0017
Indole	0.055	0.002	0.031	0.025	0.5569
2-methyl indole	0.005	0.001	0.00	0.0032	0.6849
3-methyl indole	0.001	0.001	0.00	0.001	0.7845
Acetic acid	4.339	2.918	8.9	1.854	0.3803
Propionic acid	2.035	0.587	2.515	0.9025	0.3853
2-methyl propionic acid	0.18[b]	0.00[b]	0.355[a]	0.0243	<0.0001
Butyric acid	0.835	0.211	1.485	0.8355	0.6154
3-methyl butyric acid	0.0011[b]	0.00[b]	0.0192[a]	0.003	0.0003
Pentanoic acid	0.0024	0.00	0.00	0.0032	0.7876
Undecane	0.209	0.003	0.123	0.1546	0.5467
Dodecane	0.256	0.004	0.338	0.2656	0.2943
Dimethyldisulfide	0.00	0.0014	0.00	0.001	0.2692
Nonane	0.014	0.00	0.00	0.0104	0.4569
Tridecane	0.061[b]	0.00[b]	0.214[a]	0.063	0.0306
Tetradecane	0.083	0.115	0.146	0.1051	0.7581

[1] Chemical compounds included in the stock standard solution but were not detected in significant concentrations included carbon disulfide, ethanethiol, butyrolactone, nonadecane, 4-methyl phenol, 3-methyl phenol, 4-ethyl phenol, 2,6-bis(1,1) dimethyl phenol, dimethylamine, pentane, pentane, 1-decane, 2,5 dimethylpyrazine, 2-methyl-6(1-propenyl) pyrazine, and trimethylpyrazine.

[2] Fecal samples collected from five maned wolves (n=5).

[3] Fecal samples collected from four domestic dogs (n=4).

[4] Fecal samples collected from one clouded leopard (n=1).

[5] Weighted standard error of the mean.

[a,b] Means within a row with different superscript letters differ ($P<0.05$).

Table 3-2: Volatile chemical compound concentrations (mM) identified in urine collected from maned wolves (MW), Mexican gray wolves (MGW), and domestic dogs (DD).

Chemical Compound (mM)[1]	Species			SEM[5]	P value
	MW[2]	MGW[3]	DD[4]		
Butyrolactone	0.00	0.76	0.00	0.571	0.1269
Decane	0.004	0.043	0.015	0.015	0.2064
Nononal	0.065	0.031	0.051	0.0467	0.8838
Phenol	0.00[b]	0.0037[a]	0.00[b]	0.002	0.0226
Acetic acid	0.883	0.246	0.00	0.598	0.6966
Undecane	0.018	0.019	0.069	0.002	0.1872
Dodecane	0.031	0.054	0.16	0.026	0.1322
Nonane	0.00	0.012	0.011	0.0055	0.288
1-decane	0.00	0.0044	0.0005	0.0027	0.5318
Tridecane	0.092	0.122	0.1	0.036	0.8363
Tetradecane	0.146	0.107	0.121	0.0367	0.7656
2,5-dimethylpyrazine	3.753[a]	0.00[b]	0.00[b]	0.0413	<0.0001
2 methyl-6-(1-propenyl) pyrazine	0.0044	0.00	0.00	0.003	0.5875
Trimethylpyrazine	0.0057	0.00	0.00	0.0028	0.3548

[1] Chemical compounds included in the stock standard solution but were not detected in significant concentrations included carbon disulfide, ethanethiol, nonadecane, 4-methyl phenol, 3-methyl phenol, 4-ethyl phenol, 2,6-bis(1,1) dimethyl phenol, indole, 2-methyl indole, 3-methyl indole, C3-propionic acid, 2-methyl propionic acid, C4-butyric acid, 3-methyl butyric acid, pentanoic acid, dimethylamine, dimethyldisulfide, and pentane.

[2] Urine samples collected from seven maned wolves (n=7).

[3] Urine samples collected from nine Mexican gray wolves (n=9).

[4] Urine samples collected from four domestic dogs (n=4).

[5] Weighted standard error of the mean.

[a,b] Means within a row with different superscript letters differ ($P<0.05$).

Table 3-3: Volatile chemical compound concentrations (mM) identified in air samples collected from maned wolves (MW), Mexican gray wolves (MGW), clouded leopards (CL), and black-footed ferrets (BFF).

Chemical Compound (mM)[1]	Species				SEM[6]	P value
	MW[2]	MGW[3]	CL[4]	BFF[5]		
Carbon disulfide	0.016	0.047	0.00	0.00	0.0221	0.5655
Butyrolactone	0.175	0.00	0.00	0.00	0.311	0.8427
Decane	1.131	0.059	0.061	0.071	0.8755	0.8186
Nononal	1.123	0.626	0.121	0.247	0.6188	0.2904
Phenol	0.0094	0.042	0.0036	0.00	0.0069	0.5589
Acetic acid	1.98	0.00	0.296	0.00	1.1526	0.291
Propionic acid	0.035	0.00	0.00	0.00	0.0441	0.7056
Undecane	1.754	0.139	0.076	0.233	1.084	0.7409
Dodecane	0.419	0.274	0.033	0.136	0.2213	0.2681
Nonane	0.046	0.096	0.00	0.00	0.0342	0.1672
1-decane	0.0017	0.0087	0.00	0.00	0.0042	0.8427
Tridecane	0.201	0.031	0.023	0.048	0.1714	0.5946
Tetradecane	0.061	0.058	0.03	0.011	0.0415	0.4022
2,5-dimethylpyrazine	0.0019	0.00	0.00	0.00	0.0034	0.8427

[1] Chemical compounds included in the stock standard solution but were not detected in significant concentrations included ethanethiol, nonadecane, 4-methyl phenol, 3-methyl phenol, 4-ethyl phenol, 2,6-bis(1,1) dimethyl phenol, indole, 2-methyl indole, 3-methyl indole, 2-methyl propionic acid, C4-butyric acid, 3-methyl butyric acid, pentanoic acid, dimethylamine, dimethyldisulfide, pentane, 2-methyl-6-(1-propenyl) pyrazine, and trimethylpyrazine.

[2] Air samples collected from five maned wolves (n=5).

[3] Air samples collected from three Mexican gray wolves (n=3).

[4] Air samples collected from four clouded leopards (n=4).

[5] Air samples collected from two black-footed ferrets (n=2).

[6] Weighted standard error of the mean.

[a,b] Means within a row with different superscript letters differ ($P<0.05$).

3.5 Discussion

Based on the results obtained from this preliminary study, there does not appear to be a sulfur-containing volatile compound that is unique to maned wolf urine or feces that could be responsible for the odor that is present in this species. Some species differences in urine and fecal volatile compounds were highlighted, including high levels of phenol, a common metabolite found in the environment and in excreta, in the clouded leopard (feces) and Mexican gray wolf (urine) (Liu et al. 2005). Interestingly, according to these data, the presence of pyrazine-based compounds in maned wolf urine is unique when compared to the urine and feces of other canids (Mexican gray wolf, domestic dog), a felid (clouded leopard), and a mustelid (black-footed ferret). Unlike many of the other compounds where significant concentrations appeared only sporadically (in a small percentage of samples collected), 2,5-dimethylpyrazine was consistently present in significant concentrations in all of the maned wolf urine samples.

Pyrazines are a group of compounds which are widespread in nature and are responsible for many powerful odors (Guilford 1987). Most frequently reported is the use of pyrazines by numerous insect species (Brophy 1989). For instance, 3-ethyl-2,5-dimethyl pyrazine and other similar pyrazine compounds are used as trail pheromones by several ant species (Cross 1979, Evershed 1982, Attygalle and Morgan 1984)). Pyrazines are also widespread in aposematic insects, the toxic alkaloids of which are advertised by bright warning coloration (Moore and Brown 1981, Rothschild et al. 1984, Abassi 1998), as well as in toxic plants such as poppies, milkweeds, nettle, and ragwort (Rothschild et al. 1984). Pyrazines have also been

found in several mammalian species, including the urine of coyotes (Murphy et al. 1978), the feces of rabbits (Goodrich et al. 1981), and the scent glands of the beaver (Maurer and Ohloff 1976).

Aldrich (1996) proposed that pyrazines were used as a universal warning odor, "equivalent to the color red as a visual warning signal". Other researchers have stated a similar hypothesis (Guilford 1987, Kaye 1989, Rowe 1999). The role of pyrazines for this purpose may correlate well with the normal social behavior of the maned wolf. Maned wolves, unlike North American wolves such as the gray wolves which travel in large packs, are relatively solitary. Monogamous male and female pairs defend large territories of up to 10 square miles (Dietz 1984, 1987). They deposit their feces at regular intervals around the perimeter of their territories, usually on elevated areas such as on rocks, dirt piles, and termite mounds. The odor of their urine was also found to be prevalent along perimeter trails (Dietz 1984). This conspicuous placement of feces is a characteristic maintained by captive maned wolves, which have also been noted to commonly urinate over their feces following defecation (Kleiman 1972). Therefore, the deposition of urine and feces by the maned wolf appears to play an important role in their social structure, and in combination with a strong odor from pyrazine compounds, may function to alert conspecifics and warn them not to enter the territory.

Another potential function of pyrazine odors in plants and animals that has been proposed by some researchers is to evoke memory of a situation or environment and to potentiate learning and recall of aposematic signals (Guilford 1987, Woolfson and Rothschild 1990, Herent et al. 1995). Many of the pyrazine compounds studied,

in addition to having a low olfactory threshold, also are extremely persistent (Guilford 1987). Thus, in addition to functioning as an immediate warning, the territorial marking of maned wolf territories with pyrazine compounds may function as a "reminder" to conspecifics, as well as to the wolves defending the area, of the territorial boundaries, similar to theories concerning trail marking with pyrazines in ants (Woofson and Rothschild 1990).

In conclusion, it is not likely that the strong odor of maned wolf urine and feces stems from a sulfur-containing compound and thus there does not appear to be a relationship with the high incidence of cystinuria of captive specimens. The isolation of pyrazines from the urine of all of the maned wolves tested and not from the other canids, suggests that these compounds may play an important role in the chemical communication of this species. Based on the hypothesized roles of pyrazines as olfactory message in other species and the social structure of the wild maned wolf, it is plausible that there is an important role for these compounds in territorial marking and defense. Further research in this area may include determination of gender differences in the presence or amounts of these compounds, investigation of the presence and prevalence of these compounds in maned wolves on different diets and in maned wolves in the wild, and possible interaction of pyrazine compounds with other compounds in the urine to produce an odor that is unique to this species.

3.6 References

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CHAPTER 4: Rate of Passage and Apparent Digestibility of Two Diets in the Maned Wolf (*Chrysocyon brachyurus*) and Domestic Dog (*Canis lupus*)

4.1 Abstract

The purpose of this study was to determine the digestibility of two diets manufactured for prevention of cystinuria-related clinical disease in maned wolves, as well as the rate of passage of these diets in the maned wolf, and compare these results to the digestibility and rate of passage of the same diets in domestic dogs. The experiment consisted of two diets (maintenance and experimental), two species (domestic dog and maned wolf) arranged as a 2x2 factorial design. For the rate of passage study, titanium dioxide (Ti, 5g/kg diet) was administered as a pulse dose and total collection of feces was performed for 50 hours. There was no effect of diet or species on initial Ti recovery time (IRT), the amount of Ti recovered at IRT, or the time to reach 50% of Ti excretion in the feces. Maned wolves had lower total percent recovery ($P<0.01$) of Ti than dogs and shorter time to last recovery of Ti in the feces ($P<0.05$). For the digestibility study, in which chromium oxide was used as an indigestible dietary marker, fecal samples were collected following 14 days of being fed the diets and then analyzed for dry matter, energy, protein, and minerals. Crude protein, calcium, phosphorus, and zinc apparent digestibilities were unaffected by species or diet ($P>0.05$). Apparent digestibility of dry matter ($P<0.05$) and energy ($P<0.01$) were higher in the experimental diet and in the dogs. Both dogs and wolves fed the maintenance diet exhibited a greater apparent retention of copper, iron, and magnesium ($P<0.01$). Dogs had a higher apparent retention of magnesium and sodium than the wolves.

4.2 Introduction

The maned wolf, a rare South American canid, evolved from a general canid ancestor that crossed the Panama landbridge during the early Piocene era (Langguth 1975). This species has many very unique physical, behavioral, and likely physiological characteristics. Very little research has actually been performed on the maned wolf, and thus the domestic dog is often used as a comparison in areas where research is lacking. For instance, the captive maned wolf has historically been fed like a carnivore, with early diets being composed entirely of raw meat. Subsequent manufactured diets included primarily felid diets, with a switch in more recent years to diets formulated for canids (Allen et al. 1996). Although the general diet composition of the maned wolf in the wild has been estimated (Dietz 1984, Motta-Junior et al. 1996), no nutrition studies have been performed on wild maned wolves or on typical diets of wild maned wolves. Therefore, captive diets continue to be developed and modified based on domestic canid formulations.

For much of its history in captivity, the maned wolf has been known to be generally unthrifty, with common problems including poor body condition, chronically soft stools, rapid passage of ingesta, gingivitis, and cystinuria. The high prevalence of cystinuria in the captive population prompted the development of a new experimental diet with the purpose of reduction and prevention of life-threatening clinical complications secondary to cystinuria, without the use of pharmacological agents. Because the composition of the new diet for maned wolves is altered from their normal maintenance formula, it is important to assess the availability of nutrients in this new diet. The digestibility of a diet is a measure of the biological

availability of its constituent nutrients to the animal. Digestibility is affected by numerous factors, including the chemical composition of the feed, chemical composition of other foods in a mixed ration, the quality of the feed ingredients, the method of preparation or processing of the feed, animal and environmental factors, and level of feeding.

One animal factor that may have a significant effect on diet digestibility in monogastric animals is rate of passage. Rate of passage, or intestinal transit time, is defined as the time taken for a meal to pass from the mouth to the anus. Rate of passage may vary with diet composition (Mateos and Sell 1981, Almirall and Esteve-Barcia 1994). Rate of food passage through the digestive tract may influence the amount of nutrients derived from diets by altering the duration of ingesta exposure to the digestive enzymes and to the absorptive surfaces, as well as potentially through changes in the microbial flora of the intestines and alterations in food intake ability (Maner et al. 1962, Kass et al. 1980, Mateos and Sell 1981, Mateos et al. 1982, Krogdahl 1986). The captive maned wolf is generally thought to have a very rapid rate of digesta passage through the gastrointestinal tract, especially after experiencing stress, manipulation, or dietary changes (Brady and Ditton 1979, Bush 1980, Barboza et al. 1994), although no controlled studies have been done. Mean retention times of digesta in numerous other species have been reported, including 10-16 hours in Southern elephant seals (Krockenberger and Bryden 1994), 22 hours in dogs, 15 hours in the raccoon, 13 hours in the cat (Warner 1981), and 36 hours in the pig (Ishikawa and Sugimure 1973, Kidder and Manners 1978).

The goals of this study are: 1) to determine the rate of passage of digesta in the maned wolf on the commercially available maintenance diet as well as on the new experimental diet, 2) to assess the availability of specific nutrients to the maned wolves in the new experimental diet as compared to the previous maintenance formula, 3) to determine if rate of passage and nutrient digestibility of these diets in maned wolves differs from that of domestic canids.

4.3 Materials and Methods

4.3.1 *Animals*

The study was performed on six adult maned wolves (three males and three females) maintained at the National Zoological Park's Conservation and Research Center in Front Royal, VA as well as on six beagles (four males and two females) maintained at a Land-O-Lakes animal research facility (13651 Donohoo Road, Kansas City, Kansas 66109). The dogs were housed individually in indoor concrete pens. Wolves were housed in enclosures with an outdoor region as well as a concrete indoor enclosure with a den. Details regarding den and enclosure parameters have been previously published by Brady and Ditton (1979). All wolves were maintained individually and were confined to the indoor enclosures during the rate of passage study. For the digestibility study, all wolves were allowed access to outdoor pens, and four of the wolves were allowed to remain in mated pairs. All animal procedures were approved by the institutional Animal Care and Use Committee where the animals were housed.

4.3.2 *Diets*

All animals were tested on two experimental diets which had been developed in an effort to reduce clinical complications secondary to cystinuria. Diets were manufactured by Purina Mills Inc. (St. Louis, MO) and were extruded into a medium-sized kibble. The experimental diets were formulated to be isocaloric and isonitrogenous, differing only in their protein sources and sodium level (Table 4-1). The first experimental diet ("meat-based") had approximately 40% of its protein derived from animal sources while the second experimental diet ("plant-based") had only 10% of its protein derived from animals sources. The second difference was in the sodium:potassium ratio with the plant-based diet formulated to have a lower ratio as compared to the meat-based diet.

Table 4-1: Formulated ingredient and nutrient composition in two experimental diets.

Ingredient	Maintenance Diet	Experimental Diet
	% as fed	
Meat Meal	9.58	0.00
Low Ash Poultry Meat Meal	2.00	0.00
Dried Whey	0.50	0.50
Brewer's Yeast	2.00	2.00
Beef Digest[1]	3.00	3.00
Dehulled Soybean Meal	11.81	21.50
Corn Gluten Meal	0.00	3.45
Soy Protein Concentrate	0.00	0.20
Rice Flour	25.00	25.00
Corn Flour	9.35	0.00
Poultry Fat	6.00	6.00
Bleachable Fancy Tallow	6.88	8.47
Soy Oil	0.50	0.50
Ground Beet Pulp	4.00	11.21
Apple Pomace	7.00	7.00
Ground Soy Hulls	6.44	2.77
Tomato Pomace	2.50	2.50
Sodium Chloride	0.35	0.39
Lysine	0.00	0.02
Other	3.09[2]	5.49[3]
Formulated Nutrient Concentrations (analyzed concentrations in parentheses)		
Protein, %	18.50 (18.85)	18.50 (17.85)
Fat, %	16.00 (17.2)	16.00 (16.9)
Crude fiber, %	6.5 (6.1)	6.5
Neutral detergent fiber, %	13.77 (19.2)	15.69 (20.9)
Acid detergent fiber, %	9.38 (13.95)	10.01 (16.6)
Insoluble fiber, %	(14.8)	(16.9)
Soluble fiber, %	(3.25)	(4.32)
Metabolizable Energy, kcal/kg	3530	3530
Methionine, %	0.26 (0.30)	0.27 (0.29)
Cystine, %	0.21 (0.25)	0.22 (0.27)
Taurine, %	0.30 (0.30)	0.30 (0.295)
Lysine, %	0.95 (1.04)	0.98 (1.05)
Sodium, %	0.50 (0.37)	0.20 (0.17)
Potassium, %	0.60 (0.41)	0.80 (0.48)
Calcium, %	1.39 (1.24)	1.30 (1.03)
Phosphorus, %	0.80 (0.78)	0.80 (0.62)
Magnesium, %	0.124 (0.124)	0.137 (0.110)
Iron, %	0.41 (0.40)	0.33 (0.34)
Manganese, %	0.057 (0.079)	0.058 (0.060)
Zinc, %	0.18 (0.084)	0.177 (0.142)
Copper, %	0.013 (0.010)	0.013 (0.010)
Average kcal per gram of diet:	(4639)	(4825)
Percent (%) of the protein in the diet coming from:		
Animal sources:	43.8	5.3
Plant sources:	56.2	94.7

[1] A palatability enhancing liquid ingredient with less than 10% dry matter.

[2] Contains (percent as fed): dicalcium phosphate 1.053, calcium carbonate 0.282, pyridoxine (1%) 0.144, choline chloride (70%) 0.141, menadione (2900 ppm) 0.103, vitamin D_3 (7500 IU/g) 0.053, biotin (0.1%)0.050, vitamin E (500 IU/g) 0.45, vitamin A (27240 IU/g) 0.037, ferrous sulfate (31%) 0.030, calcium iodate (60%) 0.025, zinc oxide (72%) 0.022, L-lysine 0.022, ethoxyquin 0.018, selenium (0.06%) 0.015, calcium pantothenate (17.6 g/kg) 0.013, folic acid (2%) 0.013, thiamin (10%) 0.012.

[3] Contains (percent as fed): dicalcium phosphate 2.713, calcium carbonate 0.172, pyridoxine (1%) 0.142, choline chloride (70%) 0.145, menadione (2900 ppm) 0.103, vitamin D_3 (7500 IU/g) 0.53, biotin (0.1%) 0.05, vitamin E (500 IU/g) 0.045, vitamin A (27240 IU/g) 0.037, ferrous sulfate (31%) 0.030, calcium iodate (60%) 0.025, zinc oxide (72%) 0.022, ethoxyquin 0.018, selenium (0.06%) 0.015, calcium pantothenate (17.6 g/kg) 0.013, folic acid (2%) 0.013, thiamin (10%) 0.012.

4.3.3 *Experimental Design*

4.3.3.1 General

The study was performed with both the dogs and maned wolves at the same time of the year but in different geographical locations, as specified previously. The same diet batches and protocols were used for both species. The study was designed as 2x2 factorial arrangement of two diets (maintenance and experimental) and two species (dog and wolf). The diets were tested using a split plot design, with diets being equally represented within each period and no animal being tested on the same diet twice.

Each of the two diets was randomly assigned to a group of animals within a time-period: one diet in the first period and the other diet in the second period. In each time period of 16 days, the first two days were used for the rate of passage study and the remaining 14 days constituted the digestibility study. Prior to the initiation of the study all animals were gradually transitioned to the diet on which they were to be tested in the first time-period. In addition, the two time-periods were separated by nine days during which the animals were transitioned to the diet on which they were to be tested during the second time-period.

4.3.3.2 Rate of Passage

Titanium dioxide (TiO_2), at a dosage of 5g per kg of diet fed, was placed in gelatin capsules and administered orally as a bolus to each animal at the beginning of each experimental phase. The TiO_2 dose was calculated on the previous day's food intake amount for each animal. The capsules were manually administered to the

domestic dogs while those for the maned wolves were administered by hiding the capsules in small (≤25g) mice. For 48 hours following the administration of the TiO_2 bolus, all feces were collected, placed in a plastic bag, and frozen until analysis. Diurnal defecation times were recorded and feces collected at the time of defecation. Video cameras in enclosures were used to record and estimate nocturnal defecation times and nocturnal feces were collected the following morning and identified based on videotaped location in the enclosure. Feces were collected for 50 hours after the animals were dosed, regardless of whether the animal had defecated within the last few hours from the end point.

4.3.3.3 Apparent Digestibility

At the end of the rate of passage experimental period, the animals remained on the same experimental diet that they were on in the rate of passage study, however the diets used in the digestibility trial contained 2.5g/kg of chromic oxide (Cr_2O_3). Animals were maintained on this marker-containing diet for the next fourteen days, and representative fecal samples were collected from each animal on the last two days of each experimental phase. Samples were frozen until analysis.

4.3.4 *Sample Analysis*

All samples were freeze-dried and then finely ground with a mortar and pestle.

4.3.4.1 Rate of Passage

Samples were analyzed for titanium (Ti) by a colorimetric method (Peddie et al. 1982). Briefly, 0.1g of sample was ashed for 13 hours at 580°C, then cooled. Sulfuric acid H_2SO_4 (7.4M) (10mls) was added to the ashed samples then gently boiled for approximately 60 minutes until the sample was completely dissolved. The cooled sample was then transferred into a beaker containing 25mls of distilled water. The contents of the beaker were filtered (Whatman #541) into a 100ml volumetric flask. Hydrogen peroxide (H_2O_2 30%) (20mls) was added to the volumetric flask and the flask filled to volume with distilled water. The sample was mixed well, transferred to a test tube, and absorbance read at 410nm.

4.3.4.2 Apparent Digestibility

Freeze-dried ground fecal samples were digested in a mixture of nitric and perchloric acid at 100°C for four hours, and then analyzed for chromium (Cr) (Saha and Gilbreath 1991). They were also analyzed for moisture (AOAC 1995a), nitrogen (combustion method) (AOAC 1995b), gross energy (adiabatic bomb calorimetry) (AOAC, 1980), and minerals (Anderson 1996).

4.3.5 *Statistical Analysis*

The time (hours) following administration of the titanium bolus when 50% of the marker had been recovered in feces was estimated for each animal using the second-order polynomial regression analysis model: $y = ax^2 + bx + c$, where x is the time after dosing (hours) and y is the % of total marker dosage recovered in feces

(Snedecor and Cochran 1989). The time to 50% recovery, by animal, was used as an estimated parameter for data analysis. Data for both rate of passage and digestibility were analyzed using JMP statistical analysis program (2000) as a split plot (two periods) with diets and species (arranged as a complete 2x2 factorial) represented equally in both periods with each animal tested on each diet only once. The factors were two diets (maintenance and experimental) and two species (dog and wolf). Differences between means were considered significant when the probability (P) value was equal to or below 0.05.

4.4 Results

4.4.1 *Rate of Passage*

There was no effect of diet or species on initial recovery time (IRT), the amount of Ti recovered at IRT, or the time to reach 50% of Ti excretion in the feces (Table 3). There was also no effect of diet on the total percent of Ti recovered from the feces during the trial, however, there was an effect of species. The maned wolves had a significantly lower ($P<0.01$) recovery of Ti (average 86.69%) than the dogs (average 97.02%) (Table 4-2). There was also a species effect on the time to last recovery of Ti in the feces, with the time being significantly ($P<0.05$) shorter in the wolves than in the dogs (Table 4-2). When data on recovery of Ti at the different time periods was regressed on hours from bolus time a cubic fit ($P<0.0001$, $R^2 = 0.9041$) was found for the dogs and a quadratic fit ($P<0.0001$, $R^2 = 0.9001$) for the maned wolves (Figure 4-1).

Table 4-2: Rate of passage of the maintenance and experimental diets in beagles and maned wolves as affected by diet and/or species.

Species	Diet	Initial Ti Recovery Time (IRT) (hours)[1]	Amount Ti (mg) Recovered at IRT	Time to Recovery of 50% Ti (hours)[2]	Time to last Ti Recovery (hours)[3]	Total % Ti Recovery[4]
Dog[5]	Maintenance[6]	2.88	1.11	14.19	47.5	97.03
	Experimental[7]	3.9	6.82	11.73	47.17	97.01
Maned Wolf[5]	Maintenance	4.25	3.34	13.5	43.25	87.2
	Experimental	1.17	0.49	14.77	45.17	86.18
SEM[8]		0.6004	3.5686	1.0931	1.2384	1.248
P Value		0.3938	0.5764	0.2582	0.0859	<0.01
Main Effects (Means)						
Species	Dog	3.39	3.97	12.96	47.33	97.02
	Maned Wolf	4.21	1.91	14.13	44.21	86.69
Diet	Maintenance	3.56	2.22	13.84	45.38	92.12
	Experimental	4.03	3.66	13.25	46.17	91.6
Main Effects (P Values)						
	Species	0.2035	0.5821	0.2988	0.0202	<0.01
	Diet	0.4554	0.6989	0.5907	0.0529	0.6808
	SpeciesxDiet	0.3818	0.2604	0.1039	0.3745	0.6897

1 Hours following the initial pulse dose of the marker, titanium (Ti), that the marker was first detected in the feces.

2 The number of hours following the initial pulse dose of the marker at which 50% of the titanium dose had been excreted in the feces.

3 Hours following the initial pulse dose of titanium that the last traces of the marker were detected in the feces.

4 The percentage of the initial titanium pulse dose (mg) which was recovered from the feces.

5 Beagles (6 animals) and maned wolves (6 animals). Diets tested in a split plot design with two periods.

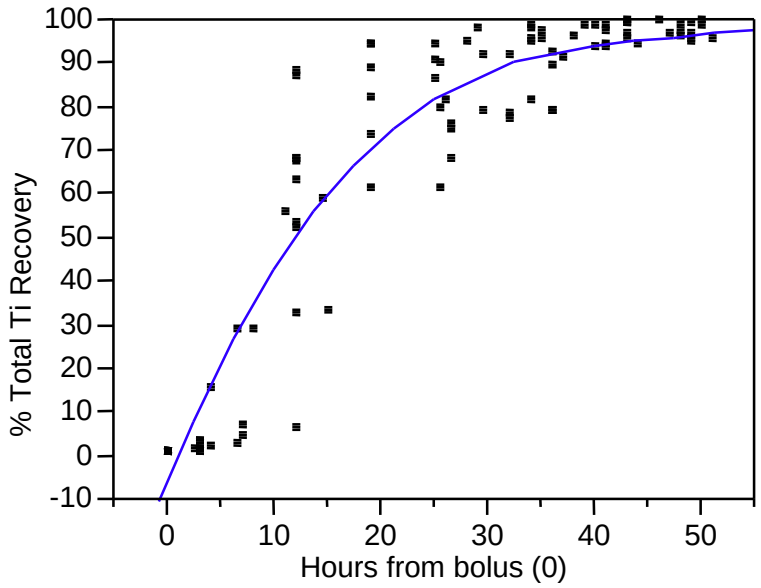
6 The maintenance diet was a commercially available diet (Mazuri Feeds®, Inc., St. Louis, MO, Maned Wolf Maintenance Diet).

7 The experimental diet, as compared to the maintenance diet, had a greater proportion of its protein from plant sources and a lower sodium to potassium ratio.

8 Standard error of the mean

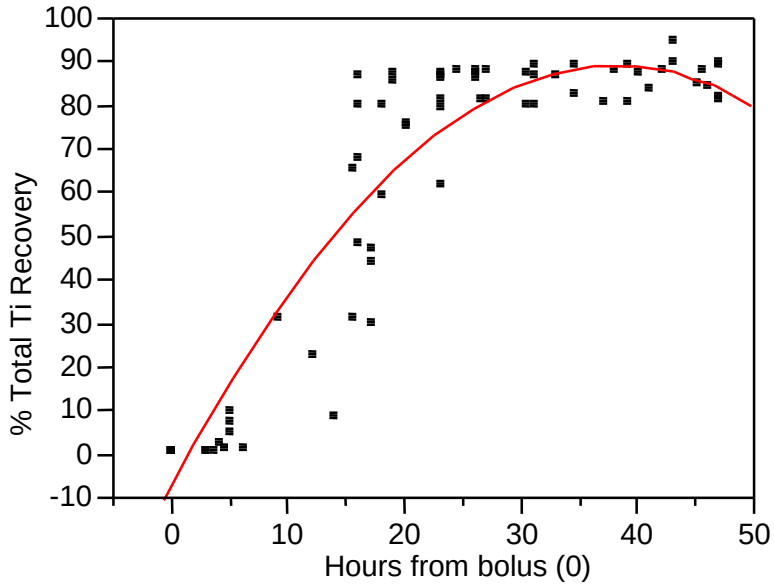
Figure 4-1: Bivariate fit of percent of total titanium recovery in the feces of dogs (**A**) and wolves (**B**) over time from the administration of the titanium bolus (t=0).

A.



Cubic fit (P<0.0001).
Percent total titanium recovery = 40.634595 + 1.6482184 hours from bolus - 0.061423 (hours from bolus-23.92)^2 + 0.0008713 (hours from bolus-23.92)^3
R^2 = 0.9041

B.



Quadratic fit (P<0.0001)
Percent total titanium recovery = 22.211487 + 2.2708667 hours from bolus - 0.0661015 (hours from bolus-21.0197)^2
R^2 = 0.9001

4.4.2 *Apparent Digestibility*

The mean apparent digestibilities of energy, dry matter, protein, fat, and selected minerals are shown in Table 4-3. There were no diet by species interactions. Crude protein (CP), calcium (Ca), phosphorus (P), and zinc (Zn) apparent digestibilities were unaffected by species or by diet treatment ($P>0.05$). The apparent digestible energy (ADE) and apparent dry matter digestibility (ADMD) were affected both by species and by diet. The experimental diet had greater ADE and ADMD than the maintenance diet (3625 vs. 3486 kcal ADE/kg diet, $P<0.01$ and 69.4% vs. 67.3% ADMD, $P<0.05$, respectively). Both ADE and ADMD were greater in dogs than in the wolves (3690 vs. 3420 kcal ADE/kg, $P<0.01$ and 71% vs. 65.7% ADMD, $P<0.01$, respectively). For copper, iron, and magnesium apparent retention, there was a strong effect of diet with animals fed the maintenance diet exhibiting a greater apparent retention than those on the experimental diet ($P<0.01$). There was also a significant effect of species on apparent magnesium and sodium retention, with higher retention in the dogs than in the wolves. The effect of species on apparent Cu retention was close to significant ($P=0.056$). Based on overall analysis of variance significance ($P<0.01$) and means contrasts, dogs had a higher apparent retention of copper and iron than maned wolves when fed the maintenance diet. Dogs on the experimental diet had a higher apparent retention of copper but not of iron.

Table 4-3: Apparent dry matter and crude protein digestibilities, apparent digestible energy, and apparent mineral retention as affected by diet and/or species.

Species	Diet	Digestible Energy (kcal/kg)	Dry Matter	Protein	Ca[1]	P[1]	Cu[1]	Zn[1]	Mg[1]	Fe[1]	Na[1]
			Digestibility (%)		----------Retention----------						
Dog[2]	Maintenance[3]	3641[b]	69.9[b]	75.6	26.7	49.4	14.6[a]	26.9	36.9[a]	16.8[a]	68.6[a]
	Experimental[4]	3740[a]	72.1[a]	76.1	23.8	49.5	9.5[c]	23	32.5[b]	9.5[c]	62.6[a]
Maned Wolf[2]	Maintenance	3331[d]	64.7[d]	71.5	22.1	49.6	11.7[b]	24.7	31.9[b]	13.8[b]	53.1[b]
	Experimental	3510[c]	66.7[c]	75.2	22.3	48	6.6[d]	24.6	26.2[c]	9.0[c]	42.1[c]
SEM[5]		29.55	0.77	1.22	26.1	2.19	1.34	2.79	1.62	1.21	3.87
P Value		<0.01	<0.01	0.058	0.55	0.94	<0.01	0.79	<0.01	<0.01	<0.01
Main Effects (Means)											
Species	Dog	3690	71	75.84	24.5	49.5	12	25.8	34.7	13.2	65.6
	Maned Wolf	3420	65.7	73.38	22.9	48.8	9.2	23.9	29	11.4	47.6
Diet	Maintenance	3486	67.3	73.55	24.4	49.5	13.1	25.8	34.4	15.4	60.9
	Experimental	3625	69.4	75.67	23	48.8	8	23.8	29.3	9.3	52.3
Main Effects (P Values)											
	Species	<0.01	<0.01	0.056	0.54	0.73	0.05	0.48	<0.01	0.08	<0.01
	Diet	<0.01	0.013	0.098	0.58	0.74	<0.01	0.46	<0.01	<0.01	0.03
	SpeciesxDiet	0.19	0.909	0.204	0.22	0.69	0.99	0.91	0.67	0.11	0.49

[1] Calcium (Ca), phosphorus (P), copper (Cu), zinc (Zn), magnesium (Mg), iron (Fe), and sodium (Na).

[2] Beagles (6 animals) and maned wolves (6 animals). Diets tested in a split plot design with two periods.

[3] The maintenance diet was a commercially available diet (Mazuri Feeds, Inc., St. Louis, MO, Maned Wolf Maintenance Diet).

[4] The experimental diet, as compared to the maintenance diet, had a greater proportion of its protein from plant sources and a lower sodium to potassium ratio.

[5] Standard error of the mean.

[a-d] Means with different superscript letters differ ($P<0.05$)

4.5 Discussion

In this study, the differences in protein composition between the maintenance and experimental diets did not have any effect on the rate of passage of nutrients. This finding appears to be in agreement with other studies where soy protein was included in the diet, especially if the protein was in a hydrolyzed rather than intact form (Zhao et al. 1997, Hill et al. 2000). Additionally, there was no significant difference between the dogs and the maned wolves for rate of passage as measured by several parameters, including time to initial recovery of the titanium marker in the feces, amount of marker obtained at initial recovery, and time to recovery of 50% of the marker in the feces. This finding contradicts previous beliefs that compared to other canids, maned wolves have an extremely rapid gastrointestinal transit time (Barboza et al. 1994). Several reports, written at a time when the wolves appeared to be being fed primarily a meat-based canid diet, describe a transit time, particularly of grass, of 20 minutes or less (Altman 1972, Brady and Ditton 1979). Passage rates were reported to be even faster when animals were stressed, resulting in diarrhea and poor body condition, and one report even describes the addition of exocrine pancreatic enzyme mixtures to the food in order to improve digestion during the short time that the food was in the intestinal tract (Bush 1980).

There was a significant effect ($P<0.05$) of species on the number of hours post-bolus that Ti was last recovered. The wolves had an earlier "last recovery" (average 44.21 hours) than did the dogs (average 47.34 hours). Several of the dogs defecated and had feces collected for analysis as late as 50 hours after the TiO_2 bolus, but many of the wolves did not defecate this close to the end of the study. Therefore,

if the wolves had been given additional time to defecate, additional Ti may have been recovered in these fecal samples, lengthening the time to last recovery of Ti as well as increasing the total percent recovery of Ti. Furthermore, unlike the dogs, the wolves were not accustomed to being confined to an indoor enclosure for an extended period of time. Although they did not appear to be stressed, their normal defecation habits may have been disrupted. Some individual wolves went long periods between defecations while others defecated more frequently. Therefore, the sample production and collection in the wolves may have been less reliable and consistent based on their temporary husbandry situation for this trial.

The total percent recovery of Ti in the feces of the dogs was high (average 97.02%), and corresponds well with Ti recovery in digestion and rate of passage studies performed in pigs (96%), chickens (97.5%) and rats (98%) (Njaa 1961, Peddie et al. 1982, Kavanagh et al. 2001). The total recovery of Ti for the wolves, however, was significantly ($P<0.01$) lower (average 86.69%). Reasons for low recovery of a fecal marker may include loss of marker in the feces, loss of marker during collection or preparation of the feces for analysis, errors in chemical measurement of the marker, and retention of the marker in the intestinal tract (Moore 1957, Njaa 1961). In this study, there were potential environmental factors that may have contributed to loss of Ti during collection. For example, the consistency of the wolf feces was softer than that of the dogs, making it more difficult to perform a total collection without leaving fecal residue on the floor or collection devices. In addition, during administration of the TiO_2 bolus to the wolves, they were occasionally observed to bite down on and rupture the capsule prior to ingestion, which may have been another

potential source of Ti loss. Alternatively, instead of loss of Ti during collection, the collection period itself may not have been long enough for the wolves, and Ti may still have been present in the gastrointestinal tract. The latter scenario seems most likely, due to the close agreement of the average percent recoveries in wolves on both the maintenance and experimental diets. If this is the case, it implies that the rate of total intestinal transit for wolves may actually be slightly longer than for the domestic dogs used in this study.

The close agreement of the various parameters used to measure rate of passage in the dogs and wolves suggest that transit time in maned wolves is similar to that in other canids when diets comparable to those tested are fed. Few studies have been performed measuring total ororectal rate of passage in domestic dogs on various diets. In normal dogs, according to Murdoch (1996), food arrives at the ileocolic junction within 5 hours of ingestion and then remains in the colon for up to 48 hours. Additional reports document total transit times of 22 hours (Warner 1981) and 19 to 34 hours (Rolfe et al. 2002), while others report times to 50% excretion for various breeds of dogs averaging 6.5-7.5 hours (Nelson et al. 1995, Allan et al. 1996, Lester et al. 1999, Weber et al. 2002). The reported rates of passage for domestic dogs show some variation, likely due to breed differences, diet differences, use of various types of markers, and differences in methodologies and definitions of rate of passage, however, the average times listed in other reports for domestic dogs appear to be shorter than those determined in the present study. This may have been due to certain components of the diets being fed to the dogs and wolves since it is well proven that diet composition can have a significant effect on rate of feed passage through the

gastrointestinal tract. Both the maintenance and experimental diets had high levels of soluble fiber. In addition to being known for a rapid passage rate, wolves fed canine and feline meat-based diets commonly had loose and even watery stool. Therefore, high levels of soluble fiber were used in the diets in an effort increase fecal bulk and improve stool quality. Dietary fiber can have various effects on gastrointestinal transit time, depending both on the type of fiber and how the fiber was processed (Smith 1980, Kritchevsky 1988, Hillemeier 1995). In many reports, insoluble fiber appears to decrease total gastrointestinal transit time, while soluble fiber tends to lengthen it (Cummings et al. 1978, Burrows et al. 1982, Stevens et al. 1988, Amirall and Esteve-Garcia 1994, Spiller 1994, Hetland and Svihus 2001).

The lack of effect of diet (maintenance vs. experimental) on protein digestibility in either the dogs or maned wolves is similar to findings in other studies in dogs where animal-protein based diets were compared to those containing vegetable or soy-based protein (Moore et al. 1980, Huber et al. 1994, Yamka et al. 2003). Several other authors have concluded that the total intestinal apparent digestibility of soy protein is in some cases slightly less than that of animal-based proteins fed to dogs (Bressani et al. 1967, Moore et al. 1980, Kendall and Holme 1982, Hill et al. 2001), however, the use of a highly processed soy product in this case (soy protein isolate) likely resulted in a high level of protein digestibility in the experimental diet (Wiernusz et al. 1995, Clapper et al. 2001).

The experimental diet resulted in higher apparent digestibilities of both energy and dry matter in both the wolves and dogs. This finding appears to be in contrast to findings in similar studies in dogs where diets containing soybean products were fed.

Several researchers observed no effect of increased soybean content in diets on dry matter or energy digestibility in dogs (Moore et al. 1980, Huber et al. 1994, Zuo et al. 1996, Murray et al. 1997, Bednar et al. 2000, Clapper et al. 2001), while others detected a significant decrease in dry matter and energy digestibility when diets containing soybean products were fed (Hill et al. 2001, Yamka et al. 2003, Swanson et al. 2004). These canine studies, however, typically used soybean meal or soy flour as the source of soy protein in their diets. Wiernusz et al. (1995) compared dry matter digestibility of four soy protein sources in dogs (soy grits, soy flour, soy protein concentrate, soy protein isolate) and documented that digestibility increased linearly as the soy product was more completely processed. Zhao et al. (1997) also found that hydrolyzed soybean had a significantly higher digestibility than intact soybean in dogs. Therefore, the use of soy protein isolate as a main source of soy protein in the diets may have resulted in a very high level of digestibility, even compared to the meat and poultry meat meal, therefore leading to the higher digestibility of DM and energy in the experimental diet.

The apparent retention of several minerals (Mg, Fe, Cu, and Na) was significantly lower in both dogs and wolves on the experimental diet as compared to the maintenance diet. This may be in part due to the presence of several antinutritional factors in soy protein, including oligosaccharides and phytate (Reddy et al. 1982, Lonnerdal 1992, Zuo et al. 1996, Clapper et al. 2001, Yamka et al. 2003, Yamka et al. 2005). In particular, the presence of phytate (*myo*-inositol hexaphosphate) in soy has been demonstrated to limit or decrease the availability of several minerals, especially in monogastric species which lack phytase in the

gastrointestinal tract (Vohra et al. 1965, Davis and Nightingale 1975, Biehl et al. 1995, Kamao et al. 2000, Traylor et al. 2001, Schoenherr et al. 2000, Hurrell 2003). A diet containing soybean protein lowered apparent magnesium absorption in rats as compared to a diet containing casein (Brink et al. 1991). In vitro studies demonstrated that phytate can form an insoluble complex with magnesium (Champagne 1988, Cheryan et al. 1983), and in vivo, lowers magnesium solubility in the intestines (Shinoda and Yoshida 1989). However, data from Brink et al. (1992) supported stimulation of fecal excretion of magnesium by phytate as a cause of reduced magnesium absorption when soybean protein was fed, rather than inhibition of absorption due to formation of insoluble complexes. There have been similar findings for iron, where phytate in soybean protein has been shown to significantly decrease iron bioavailability (Welch and Van Campen 1975, Schricker et al. 1983, Thompson and Erdman 1984, Hisayasu et al. 1992, Hurrell et al. 1992, Davidsson et al. 1994, Lynch et al. 1994, Perez-Llamas et al. 2001, Hurrell 2004). In addition to phytate, other factors in soybean protein are though to inhibit iron absorption including conglycinin and lectins (Hisayasu et al. 1992, Hurrell et al. 1992, Lynch et al. 1994). With an effect of phytate from high levels of soybean protein in the experimental diet, however, it is unusual that there is no significant effect of diet on the apparent retention of zinc, which has also been found to be negatively affected by phytate (Zhou et al. 1992, Lonnerdal et al. 1999, Kamao et al. 2000, Norii and Suzuki 2002, Egli et al. 2004). Researchers have also observed lower absorption of Cu when diets composed primarily of soybean products are fed (Waibel et al. 1964, Robbins and Baker 1980, Baker and Czarnecki-Maulden 1987, Leach et al. 1990), however

numerous studies provide evidence that this decreased bioavailability is not due to an effect of phytate as it is in magnesium and iron (Morris et al. 1984, Twinlund et al. 1985, Lee et al. 1988, Funk and Baker 1991, Lonnerdal et al. 1999, Kamao et al. 2000). The decrease in sodium digestibility in dogs and wolves on the experimental diet is likely due to some effect of the soy protein on reducing digestibility (March 1984, Hill et al. 2001), but also may reflect the fact that the experimental diet is much lower in sodium (0.17%) than the maintenance diet (0.37%).

Several of the nutrient values measured in this study were affected by species, including dry matter, energy, copper, magnesium, iron, and sodium. In all cases, there was a similar trend toward greater apparent digestibility or retention in the dogs than in the wolves. Also, based on the findings in this study of similar rate of passage between dogs and maned wolves on two different diets, the oro-rectal transit time, or total rate of food passage, was not a contributor to any of the significant species or diet effects on nutrient digestibility discussed previously.

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CHAPTER 5: Taurine Deficiency in Maned Wolves (*Chrysocyon brachyurus*) Maintained on Two Diets Manufactured for Prevention of Cystine Urolithiasis

5.1 Abstract

This study was performed in an effort to assess the long-term effects of an experimental diet vs. a commercially available manufactured diet, both intended to reduce clinical disease related to cystinuria, on the taurine status of captive maned wolves. For thirteen weeks, two pairs of maned wolves were maintained on the commercially available maintenance diet, while two individually housed wolves were maintained on the experimental diet. All six wolves, both at the beginning and at the end of the diet trial, had severely decreased plasma concentrations of taurine (as compared to the normal canine reference range of 60-120 nmol/ml) (NRC, 2003) with average taurine concentrations of 16 nmol/ml at the beginning of the study and 3 nmol/ml at the end of the study. There was no statistically significant difference in the taurine concentrations between animals on the maintenance vs. experimental diets. Both diets were subsequently supplemented with taurine at a concentration of 0.3%. All study animals were eventually switched to the taurine-supplemented version of the commercially manufactured maintenance diet and subsequent samplings were performed to monitor plasma taurine concentrations. A final sampling, performed approximately 5 months following the initiation of taurine supplementation, revealed an average taurine concentration within the target canine reference range (90.25 nmol/ml). There are numerous physiologic (e.g. possible unique metabolism and requirements for taurine in this species as compared to other

canids) and dietary factors (e.g. effects of the types and concentrations of both fiber and protein on nutrient availability, taurine metabolism, and enterohepatic circulation of taurine-conjugated bile salts; impaired taurine synthesis secondary to low cysteine availability) that could be potential contributors to the development of taurine deficiency in the maned wolves in this study. Taurine supplementation should be considered in maned wolves maintained on diets intended for reduction of cystinuria-related complications.

5.2 Introduction

The maned wolf is a rare South American canid of which a relatively small captive population is currently maintained in zoological parks across the United States. In a captive setting, the maned wolf is known for being generally unthrifty and having marginal to poor reproductive success, however, the most serious and widely recognized disorder afflicting captive maned wolves with an alarming prevalence is cystinuria (Bovee and Bush 1978, Bush and Bovee 1978, Bovee et al. 1981, Mussart and Coppo 1999). Cystinuria, which has been shown to have an autosomal recessive mode of inheritance in both humans and domestic dogs, is characterized by defective transport of the nonessential amino acid cysteine, and the dibasic amino acids lysine, ornithine, and arginine, through the epithelial cells of both the renal tubular and intestinal brush border membranes (Harris et al. 1955, Rosenberg et al. 1965, Casal et al. 1995). Excess concentrations of cysteine in the urine often result in clinical disease secondary to the formation of cystine uroliths, which, depending on their location, can predispose the patient to complications such

as urinary tract infections and renal insufficiency, as well as cause life-threatening upper and lower urinary tract obstruction (Lindell et al. 1997, Rutchik and Resnick 1997, Joly et al. 1999, Goodyer et al. 2000). Maned wolves have historically been plagued by complications related to cystinuria that have resulted in considerable morbidity and mortality (Bovee and Bush 1978, Bush and Bovee 1978, Bovee et al. 1981, Mussart and Coppo 1999). Basic therapeutic principles in the management of cystinuria include increasing the solubility of cystine in the urine (by increasing urine pH) as well as reducing urinary cystine concentration, and these objectives can be approached by urinary alkalinization, administration of thiol-containing drugs, and dietary modification (Joly et al. 1999, Ng and Streem 1999, Barbey et al. 2000).

In an effort to reduce the incidence of clinical disease from cystinuria in the maned wolf, nutritional modification has been previously researched (Boniface 1998), resulting in the development of a maned wolf diet which was made commercially available in 1998 and was fed to almost all of the maned wolves in the United States (M. Rodden, personal communication, 2001). This diet, which was characterized primarily by a moderate protein concentration, a low concentration of sulfur-containing amino acids (cysteine and methionine), and a high fiber concentration, resulted in a significant decrease in the urinary excretion of cystine, but did not result in a significant increase in the urine pH (Boniface, 1998). The solubility of cysteine in urine is highly dependent on pH. Low urine pH promotes cystine precipitation and urolith formation, while high urine pH results in increased cysteine solubility (Osborne et al. 1989, Dent and Senior 1995). In an effort to make further improvements on this diet, an experimental diet was developed which was

demonstrated to significantly raise the urine pH in the maned wolves tested (Childs–Sanford and Angel, unpublished data). In addition, the sodium (Na) concentration in this experimental diet was decreased based on human studies which demonstrated that urinary excretion of cysteine is directly correlated with urinary Na excretion, with low Na diets effectively reducing urinary cysteine excretion (Jaeger et al. 1986, Norman and Manette 1990, Peces et al. 1991, Rodriguez et al. 1995). In an effort to assess the long-term effects of this experimental diet versus the commercially available manufactured diet on the overall nutritional and health status of the captive maned wolf a long-term diet trial was initiated.

5.3 Materials and Methods

5.3.1 Animal Husbandry

All animal related activities were approved by the institutional Animal Care and Use Committee. Seven adult maned wolves were maintained at the National Zoological Park's Conservation and Research Center in Front Royal, Virginia. Three of the wolves were housed individually in enclosures with an outdoor area as well as a concrete indoor enclosure with a den. The other four animals remained in mated pairs, with one pair having an enclosure similar to that of the individual animals and the second pair having a large outdoor enclosure with access to small dens constructed of wood. Details regarding den and enclosure parameters have been previously published by Brady and Ditton (1979). One wolf died during the trial due to a gastric dilatation volvulus. Both pairs became pregnant and gave birth during the study, however the pups were presumed to be ingested by the dam and/or sire soon

after birth in both instances and it is not known if the pups were alive or not at the time of their birth. All wolves involved in the study had access to and were known to occasionally consume invertebrates as well as small mammalian and avian wildlife in their outdoor enclosures during the course of the study, which spanned from December 2001 to July 2002.

5.3.2 *Diet Trial*

There were two diets tested in this study: a commercially manufactured maned wolf maintenance kibble (Mazuri®, PMI Nutrition International, St. Louis, Missouri, 63144), referred to in this paper as the maintenance diet, and an experimental diet that differed only in the proportion of animal-based to plant-based protein as well as the sodium to potassium ratio (Table 5-1). Both diets were made using the same extrusion process and equipment so that shape and physical characteristics were similar. Approximately 40% of the protein in the maintenance diet was derived from animal sources, while the protein in the experimental diet had only approximately 5% of its protein derived from animal sources. The sodium:potassium ratio was also different between the two diets. The experimental diet had a lower ratio (0.2% Na:0.8%K) versus that in the maintenance diet (0.5% Na:0.6% K) (Table 5-1). For this long-term study, the four maned wolves that were housed in pairs were assigned to the original maintenance diet (which was the diet they had been on previously), while the three individually-housed wolves were gradually switched to the experimental diet. Animals were fed once daily in the morning and were each offered approximately 800 grams of kibble per day. All

extraneous items normally added to the diet at this facility were discontinued except for two mice (approximately 25g each) per wolf per day. The wolves were maintained on their respective diets for approximately 13 weeks.

Table 5-1: Formulated ingredient and nutrient composition of the experimental diets.

Ingredient	Maintenance Diet	Experimental Diet
	% as fed	
Meat Meal	9.58	0.00
Low Ash Poultry Meat Meal	2.00	0.00
Dried Whey	0.50	0.50
Brewer's Yeast	2.00	2.00
Beef Digest[1]	3.00	3.00
Dehulled Soybean Meal	11.81	21.50
Corn Gluten Meal	0.00	3.45
Soy Protein Concentrate	0.00	0.20
Rice Flour	25.00	25.00
Corn Flour	9.35	0.00
Poultry Fat	6.00	6.00
Bleachable Fancy Tallow	6.88	8.47
Soy Oil	0.50	0.50
Ground Beet Pulp	4.00	11.21
Apple Pomace	7.00	7.00
Ground Soy Hulls	6.44	2.77
Tomato Pomace	2.50	2.50
Sodium Chloride	0.35	0.39
Lysine	0.00	0.02
Other	3.09[2]	5.49[3]
Formulated Nutrient Concentrations (analyzed concentrations in parentheses)		
Protein, %	18.50 (18.81)	18.50 (18.55)
Fat, %	16.00 (17.2)	16.00 (16.9)
Crude fiber, %	6.5	6.5
Neutral detergent fiber, %	13.77	15.69
Acid detergent fiber, %	9.38	10.01
Insoluble fiber, %	(14.8)	(16.9)
Soluble fiber, %	(3.25)	(4.32)
Metabolizable Energy, kcal/kg	3530	3530
Methionine, %	0.26 (0.49)	0.27 (0.43)
Cystine, %	0.21 (0.34)	0.22 (0.37)
Lysine, %	0.95 (1.04)	0.98 (1.05)
Sodium, %	0.50	0.20
Potassium, %	0.60	0.80
Percent (%) of the protein in the diet coming from:		
Animal sources:	43.8	5.3
Plant sources:	56.2	94.7

[1] A palatability enhancing liquid ingredient with less than 10% dry matter.
[2] Contains (percent as fed): dicalcium phosphate 1.053, calcium carbonate 0.282, pyridoxine (1%) 0.144, choline chloride (70%) 0.141, menadione (2900 ppm) 0.103, vitamin D_3 (7500 IU/g) 0.053, biotin (0.1%)0.050, vitamin E (500 IU/g) 0.45, vitamin A (27240 IU/g) 0.037, ferrous sulfate (31%) 0.030, calcium iodate (60%) 0.025, zinc oxide (72%) 0.022, L-lysine 0.022, ethoxyquin 0.018, selenium (0.06%) 0.015, calcium pantothenate (17.6 g/kg) 0.013, folic acid (2%) 0.013, thiamin (10%) 0.012.
[3] Contains (percent as fed): dicalcium phosphate 2.713, calcium carbonate 0.172, pyridoxine (1%) 0.142, choline chloride (70%) 0.145, menadione (2900 ppm) 0.103, vitamin D_3 (7500 IU/g) 0.53, biotin (0.1%) 0.05, vitamin E (500 IU/g) 0.045, vitamin A (27240 IU/g) 0.037, ferrous sulfate (31%) 0.030, calcium iodate (60%) 0.025, zinc oxide (72%) 0.022, ethoxyquin 0.018, selenium (0.06%) 0.015, calcium pantothenate (17.6 g/kg) 0.013, folic acid (2%) 0.013, thiamin (10%) 0.012.

5.3.3 *Animal Procedures*

Four weeks prior to the start of the diet trial (November 1, 2001), animals were restrained in a squeeze cage and heavily sedated with Telazol® (zolazepam plus tiletamine, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501, USA) administered intramuscularly with a hand syringe. Blood (12 mls) was collected from the jugular vein directly into a heparinized syringe. At the end of the diet trial (March 4, 2002), a second immobilization was performed and blood was collected in an identical manner to the first immobilization.

5.3.4 *Sample Processing*

Heparinized blood samples were immediately centrifuged at 8,000rpm for 10 minutes and two mls of plasma were transferred to a separate centrifuge tube. An equal amount (two mls) of sulfasalicylic acid was added to the plasma and centrifuged at 14,000rpm for 25 minutes. The supernatant was transferred to a plastic microtainer tube and frozen at -80 ^{o}C until analysis at the completion of the study.

5.3.5 *Taurine Analysis*

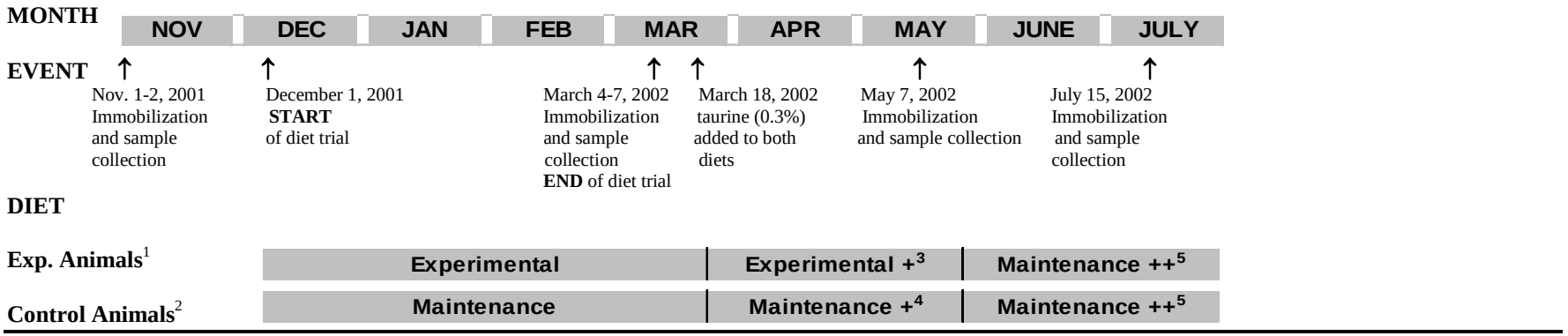
Samples were analyzed at the Amino Acid Analysis Laboratory, Department of Molecular Biosciences, University of California at Davis. Plasma taurine concentrations were determined by the use of an automated analyzer (Model 7300, Beckman Instruments, Palo Alto, CA), which utilizes cation-exchange chromatography and spectroscopic determination of a ninhydrin reaction with amino

acids to obtain measured values from a plasma sample. Norleucine was used as an internal standard to standardize the concentrations of amino acids across time.

5.3.6 *Diet Alterations and Subsequent Immobilizations*

Following the initial (November 2001) immobilizations, the three individually-housed wolves in the experimental group were gradually (over three weeks) converted from the commercial maintenance diet to the experimental diet. All animals were kept on the same diets from the start of the trial (December 1, 2001) until March 2002 (Figure 5-1). As the diet trial progressed, there were reports of decreased appetite and gradual weight loss of the wolves. Due to these clinical signs the trial was terminated in March 2002, approximately 4 weeks earlier than originally planned. Based on the taurine results from both the November and March samples, which were obtained after the March immobilization, the decision to add taurine to both of the diets was deemed necessary. Taurine was therefore added to both the experimental and maintenance diets at a concentration of 0.3% and fed to the wolves beginning March 18, 2002. This concentration of taurine was also added to all new batches of the commercial maintenance kibble produced and distributed in the United States by the manufacturer. Two subsequent immobilizations were performed to monitor the response to taurine supplementation (May 2002 and July 2002). Immobilizations, sample processing, and sample analysis were performed as described previously. Immediately following the May 2002 immobilization, all wolves were put on the maintenance formula with taurine supplementation (0.3%).

Figure 5-1: Timeline of events during a diet trial testing an experimental vs. a maintenance diet in maned wolves.



[1] n=2 (Three animals were in this group until January 10th when one animal died.)

[2] n=4 (Two mated pairs)

[3] Experimental diet plus taurine at 0.3% (started March 18).

[4] Maintenance diet plus taurine at 0.3% (started March 18).

[5] Maintenance diet plus taurine at 0.3% as well as miscellaneous food items added by the institution including (per animal) three 25g mice, ¼ can (3.3 oz.) Pedigree® Complete dog food, 6 grapes, and 30cc Mirra-Coat Liquid® (PetAg, Inc., Hampshire, IL 60140) (started May 8).

5.3.7 *Statistical Analysis*

The results of the study were analyzed as a crossover design with heterogenous variances for the time periods before and after taurine was added to the diets using the mixed procedures of SAS (1999). Fixed effects were diet and date of measurement, and wolf ID was a random effect. Pairwise comparisons using Tukey's HSD to control for experiment-wise error rate (Ott and Longnecker, 2004), and a contrast comparing average plasma taurine concentrations before and after addition of taurine to the diet, were performed. Means were considered significantly different at $P<0.05$.

5.4 Results

Plasma taurine concentrations for individual wolves at the time of each immobilization are shown in Table 5-2, and average plasma taurine concentrations from each immobilization are displayed in Table 5-3. Plasma taurine concentrations of all wolves, both at the beginning of the diet trial (November 2001) and at the end of the diet trial (March 2002), are severely deficient when compared to the normal canine reference range of 60-120 nmol/ml (Amino Acid Analysis Laboratory, Department of Molecular Biosciences, University of California at Davis). Although all animals demonstrated a decline in taurine concentrations between the first and second immobilizations, the decline was not significant ($P<0.07$) (Table 5-4). There was no significant difference in taurine levels at either immobilization date (November vs. March) between animals on the maintenance diet vs. the experimental diet, with average plasma taurine concentrations of 12.08 nmol/ml and 5.16 nmol/ml

in wolves on the experimental diet in November and March, respectively, and average plasma taurine concentrations of 17.96 nmol/ml and 2.06 nmol/ml in the wolves on the maintenance diet for these same respective months. The average plasma taurine concentration remained at the low end of the domestic canine normal range at the May 2002 immobilization, approximately 8 weeks after the diets had been supplemented with additional taurine at 0.3% of diet. At the final sampling in July 2002, approximately 16 weeks after diet supplementation with taurine, average plasma taurine for the six wolves was within the target domestic canine reference range.

Table 5-2: Plasma taurine concentrations (nmol/ml) in six maned wolves.

IMMOBILIZATION DATE	WOLF 1	2	3	4	5	6
November 2001	16.79	7.37	11.9	10.96	37.43	11.56
March 2002	7.88	2.45	1.79	1.89	2.619	1.95
May 2002	105.76	21.25	22.74	99.69	24.01	121.07
July 2002	146.68	122.65	31.34	32.82	141.65	66.38

Table 5-3: Changes in plasma taurine concentrations (nmol/ml) in maned wolves associated with several generalized changes in feeding strategy.

SAMPLING DATE	DIET	AVERAGE PLASMA TAURINE[1] (nmol/ml)	SEM
November 2001	Maintenance[2]	18.79[b3]	4.83
March 2002	Maintenance/Experimental[4]	4.03[c]	6.58
May 2002	Maintenance plus[5]	66.68[a]	20.28
July 2002	Maintenance plus/Canine Maintenance[6]	91.18[a]	20.28
P value[7]			
Diet		0.7125	
Supplemental taurine		0.0003	
P value[8]			
Date		0.0006	
Before and after[9]		0.0004	

1 Mean plasma taurine result from each of the 6 maned wolves involved in the study.

2 The maintenance diet was a commercially manufactured diet (Mazuri Feeds, Inc., Maned Wolf Maintenance). The formulated taurine concentration in this diet was 0.25%.

3 Mean plasma taurine concentration at each time period regardless of diet.

4 During the diet trial period (November 2001 to March 2002), two wolves were fed the Experimental diet, and four wolves were fed the Maintenance diet. There was no effect of diet (Experimental vs. Maintenance) on plasma taurine concentrations during the diet trial and thus the mean is for all six wolves in the study (see Table 2).

5 After the March 2002 sampling, the Experimental and commercially manufactured Maintenance diets were modified as follows: Taurine added to an analyzed concentration of 0.3% and Fe added to an analyzed concentration of 0.04%. (Two wolves were maintained on the Experimental Diet in order to participate in another study).

6 Three weeks prior to the July sampling the wolves were switched to a commercial canine maintenance diet (Purina One® dry kibble plus Pedigree® canned food).

7 Data analysis based on testing the effect of diet and the effect of taurine supplementation.

8 Data analysis performed disregarding which diet was fed since there was no effect of diet.

9 Contrast between plasma taurine values before taurine supplementation was initiated (November 2001 and March 2002) and after taurine was added to the diets (May and July 2002).

a-c Means with different superscript letters differ ($P<0.01$).

Table 5-4: Changes in plasma taurine concentrations (nmol/ml) associated with specific changes in feeding strategy during and after the diet trial.

SAMPLING DATE	DIET	AVERAGE PLASMA TAURINE (nmol/ml)	SEM
Initial[1]	Maintenance[2]	18.79[3]	4.46
Final[4]	Experimental[5]	5.16[6]	1.38
Final	Maintenance	2.06[7]	0.974

[1] Initial plasma sample taken immediately before the experiment started (November 2001). All wolves were on the maintenance diet before the start of the experiment.
[2] The maintenance diet was a commercial diet (Mazuri Feeds, Inc., Maned Wolf Maintenance).
[3] Mean of six wolves.
[4] Final sampling occurred four months after the start of the experiment (March 2002).
[5] The experimental diet was mainly a plant protein-based diet vs. the maintenance diet which was a predominantly meat-based diet.
[6] Mean of two wolves.
[7] Mean of four wolves.

5.5 Discussion

As compared to both normal canine and feline reference ranges for plasma taurine, the maned wolves in this study demonstrated a severe taurine deficiency prior to the start as well as at the completion of the diet study (November 2001 to March 2002). Although normal reference ranges for plasma taurine have not been established for the maned wolf, the dramatically low concentrations as compared to normal reference ranges for domestic species (canine reference range 60-120 nmol/ml; feline reference range 80-120 nmol/ml) (Amino Acid Laboratory, Department of Molecular Biosciences, University of California at Davis), likely do represent a true deficiency. Most authors consider plasma taurine concentrations less than 40nmol/ml as evidence of taurine deficiency in dogs (Backus et al. 2003, Delaney et al. 2003), although some suggest that less than 25nmol/ml indicates a deficiency (Kramer et al. 1995). Regardless, the plasma taurine concentrations of all six of the maned wolves involved in this study were well below these suggested critical levels.

Taurine is obtained by animals through two methods: diet and biosynthesis. Plants tend to be poor sources of taurine, so taurine is obtained from the diet primarily through ingestion of foods of animal origin (Spitze et al. 2003). In this trial, both the experimental and maintenance diets had a significant proportion of their protein from plant sources and thus dietary taurine concentrations may not have been adequate for the maned wolves. However, if this was the case, a significant effect of diet on the plasma taurine concentrations in the wolves would have been expected, since the experimental diet had 95% of its protein derived from plant sources, while the

maintenance diet had only 60% of its protein from plant sources. Furthermore, the analyzed concentrations of taurine in the diets fed to the wolves prior to taurine supplementation were typical of average commercial domestic canine extruded dry diets (Backus et. al. 2003). This would therefore imply that maned wolves could potentially have a dietary requirement for taurine, unlike the domestic dog, for which no requirement is considered necessary (NRC 2003). The taurine requirement for adult cats as published by the National Research Council (2003) is 0.4%, and no requirement is listed for the domestic dog.

Like cats, domestic canids are obligated to conjugate their bile acids to taurine, however, they are not assumed to have a dietary requirement for taurine due to their capability for taurine biosynthesis. Biosynthesis of taurine occurs primarily in the liver from cysteine and/or methionine. The conversion of these sulfur-containing amino acids to taurine is regulated by the activities of the two rate-limiting enzymes cysteine dioxygenase and cysteinesulfinate decarboxylase (Stipanuk et al. 1992a). Felids have low activity of both of these enzymes, especially cysteine dioxygenase, contributing to the essentiality of taurine in this family (Knopf et al. 1978). Typical canids have adequate enzyme activity in the liver to synthesize needed taurine under normal conditions (Hayes 1989). The presence and level of activity of these hepatic enzymes have not been studied in the maned wolf. Therefore, it is possible that the maned wolf, like the cat, has a high dietary requirement for taurine based on an inability to adequately synthesis taurine in the liver. On the other hand, if maned wolves, like dogs, do have adequate hepatic enzyme activity for taurine biosynthesis, the low concentrations of both cysteine and

methionine in the diets tested in this experiment could potentially have decreased the ability of the wolves to synthesize adequate amounts of taurine. Cystinuric humans and domestic dogs have been documented to have low plasma and urine taurine concentrations, and it is hypothesized that this is primarily due to decreased synthesis capabilities due to low plasma cysteine concentrations (Martensson et al. 1990, Sanderson et al. 2001a, Sanderson et al. 2001b). Furthermore, under conditions of low substrate availability for the taurine synthesis reaction, formation of alternate products may be favored. For example, in rat hepatocytes, glutathione formation is favored over taurine synthesis during conditions of low cysteine availability (Stipanuk et al. 1992b).

Dietary factors may have also resulted in interference with normal taurine metabolism. This has been demonstrated in numerous species, including recent evidence published on domestic canids in which dogs maintained on commercial maintenance diets, especially those containing rice bran or whole grain rice with lamb meal, exhibited low plasma taurine concentrations (Fascetti et al. 2003, Backus et al. 2003). The two main routes of taurine removal from the tissues include the conjugation of bile acids and fecal excretion. Approximately 95% of conjugated bile acids are normally reabsorbed in the distal ileum via high affinity receptors and returned to the liver through the portal circulation, completing the cycle of enterohepatic circulation. Taurine that becomes deconjugated from bile acids or that is unabsorbed from the diet is lost through fecal excretion. Numerous dietary factors have been demonstrated to have effects on the enterohepatic circulation and fecal loss of taurine, including the type of dietary protein. In this experiment, soybean protein

isolate was utilized to supply the majority of the plant-based protein in both the maintenance and experimental diets. Soybean protein has been demonstrated to result in increased taurine loss, both through enhancement of the microbial degradation of bile acids as well as the acceleration of cholecystokinin-mediated turnover of bile acids (Morris et al. 1994, Kim et al. 1995, Backus et al. 1995). Furthermore, soybean proteins have been shown to have the ability to bind and sequester bile acids within the intestinal tract through the formation of hydrophobic peptides, thus preventing their reabsorption and recirculation to the liver (Huff and Carol 1980, Makino et al. 1988, Choi et al. 1989, Benyen 1990, Iwami et al. 1990, Sugano et al. 1990, Hickman et al. 1992a,b). Fiber has also been demonstrated to interfere with the enterohepatic circulation of bile acids and thus taurine. Both the maintenance and experimental maned wolf diets were very high in fiber, primarily soluble fiber. In rats, soluble dietary fiber has been demonstrated to increase fecal loss of bile salts and decrease hepatic concentrations of taurine as compared to diets containing insoluble fiber (Ide et al. 1989, Buhman et al. 1998, Ginnett et al. 2003).

Taurine deficiency has been reported in several animal species including cats, dogs, foxes, and exotic felids (Sturman 1992, Moise et al. 1991, Kittleson et al. 1997, Kramer et al. 1995, Hayes and Trautwein 1989, Howard et al. 1987, Ofri et al. 1996). Taurine, a sulfur-containing β-amino acid, is one of the most abundant free amino acids in the body and has important functions in virtually all body systems. In addition to its role in normal bile salt function, taurine is essential for normal retinal, cardiac, neurologic, reproductive, immune, and platelet function, and also functions as an antioxidant (Sturman and Messing 1990, Hickman et al. 1992, Chapman et al.

1993, Stapleton et al. 1998, Mankovskaya et al. 2000, Lima et al. 2001, Miglis et al. 2002, Militante and Lombardini 2002, Schuller-Levis and Park 2003). Therefore, the effects of a taurine deficiency are likely widespread in multiple organ systems. The clinically apparent results of taurine deficiency are most well described in the domestic cat, and typically include central retinal degeneration (Barnett and Burger 1980, Hayes et al. 1975), dilated cardiomyopathy (Pion et al. 1987), and reproductive problems (Sturman 1986). There were no physical examination findings or clinical signs suggestive of central retinal degeneration or dilated cardiomyopathy in the maned wolves involved in this study. Interestingly, however, poor reproduction has been a chronic problem of maned wolves in captivity in the United States, especially in recent years (M. Rodden, personal communication). For example, in the 2001-2002 breeding season, there were only two puppies born that lived, and ironically, these puppies were born at the only zoo in the United States at that time that did not feed the commercial maintenance diet to their maned wolves. In contrast, during the following breeding season (2002-2003), after supplementation of the diet with taurine, a record twenty-four puppies were born. During pregnancy in mammals, taurine is accumulated in the maternal tissues in preparation for delivery through the placenta and milk to the fetus and neonate, as taurine is an essential amino acid for the offspring during the perinatal period (Dieter et al. 1993, Aerts and VanAssche 2002). Domestic cats with taurine deficiency exhibit increased fetal resorption and stillbirths, low birth weight of offspring, and decreased survivability of liveborn kittens. In those kittens that survive, there is an increased incidence of developmental defects including retinal degradation, delayed cerebellar cell division and migration,

and abnormal cortical development (Sturman et al. 1986, Sturman 1988). The reproductive problems recognized in captive maned wolves in the past have not been thoroughly characterized, but include poor puppy survival, killing of the puppies by the dam, and reduced fertility. It is therefore highly possible that a major contributor to the poor reproductive success of captive maned wolves in the United States has been taurine deficiency.

Although plasma taurine concentrations drop to low concentrations in as quickly as four weeks in domestic cats maintained on diets deficient in taurine, in both cats and dogs, clinical signs related to the deficiency may take months to years to develop (Pion et al. 1992, Pion et al. 1987, Sanderson et al. 2001). Therefore, additional clinical manifestations of taurine deficiency in the maned wolves may have developed if the severe deficiency had continued longer. The severity of the taurine deficiency in the maned wolves may have been exacerbated by the fact that supplemental food items normally added to the maintenance kibble at this facility, which include prey items such as mice which are high in taurine, were removed from the diet for the purpose of the study. The plasma taurine concentrations of the wolves on the commercial maintenance diet (without taurine supplementation) including these supplemental items has not been evaluated, although the tremendous improvement in reproductive success in the breeding season following taurine supplementation in comparison to the poor reproductive success in years prior to this study, imply that taurine deficiency may have existed prior to this study.

Based on extremely low plasma taurine concentrations and the response of these concentrations to taurine supplementation, a taurine deficiency was documented

in the maned wolves involved in this study. The findings in this study may imply that maned wolves have a dietary requirement for taurine, and also suggest that diet, especially protein and fiber type and concentration, may have a significant effect on plasma taurine concentrations in this species. The circumstantial evidence involving improved reproductive success of maned wolves in the United States following taurine supplementation of the diet supports a role of taurine in the poor reproductive success of the maned wolf, and also may indicate that the clinical effects of taurine in this species are primarily reproductive. Future research on taurine status and the physiology of taurine metabolism in maned wolves is indicated. Based on the data from this study, the authors recommend that all maned wolves maintained on low protein or otherwise modified diets intended for the prevention of cystinuria-related clinical disease be provided with supplemental taurine at concentrations recommended for domestic felids.

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Chapter 6: Summary and Conclusions:

This research project was designed in an effort to improve the overall nutritional status of the captive maned wolf as well as reduce the risk of cystinuria-related complications without the use of pharmacologic agents. We hypothesized that maned wolves in captivity have historically experienced significant morbidity and mortality from this disease due primarily to an inappropriate diet which promoted excess cystine excretion and decreased cystine solubility secondary to a low urine pH. Increasing the proportion of plant-based protein in the diet, independent of sulfur amino acid content did result in a significant increase in average urine pH in maned wolves, however the increase did not appear to bring the urine pH into a range that would maximize cystine solubility. The change in protein source, however, also resulted in a decrease in the severity of cystine crystalluria and a lower score on the cyanide nitroprusside test. Based on the data from this study it was not possible to demonstrate a significant effect of low dietary sodium on decreasing urine cysteine excretion. This may have been due, in part, to the inability to appropriately monitor urine cysteine excretion over a 24 hour period in this species in order to eliminate or reduce the impact of environmental factors or individual variation on single results.

In addition to having an alarmingly high prevalence of cystinuria in the captive population in the United States, maned wolves are known for having a peculiar and powerful odor to their excreta. The possibility of this odor being caused by a sulfur-containing compound that is in some way related to cystinuria was made less likely through a study of the volatile compounds in maned wolf urine and feces,

in which no such sulfur-containing compound was detected. Instead, a high concentration of a pyrazine compound was found in all of the maned wolf urine samples, but was not detected in urine samples from Mexican gray wolves or domestic dogs. Pyrazine compounds have been hypothesized in other species, especially insects and plants, to serve as an alert or warning, and therefore may play an important role in the marking and defense of the large territories maintained by single or pairs of maned wolves in the wild.

Since there is frequent reference in the literature to an extremely rapid intestinal transit in captive maned wolves, and rate of passage can have a significant effect on nutrient digestibility, these two factors were investigated using the commercially available maned wolf maintenance diet and one of the experimental diets from our first study. No significant differences in several parameters commonly used to measure rate of passage were detected between maned wolves and dogs. The effects that were seen on the amount of time until the marker was last measured, as well as total percentage of marker recovered, could both be caused by continued retention of marker in the digestive tracts of the wolves. This implies that if truly different from the dogs, the rate of passage of wolves on these diets may actually be slightly longer. Furthermore, the similar intestinal transit time between the maned wolves and dogs indicates that rate of passage did not play an important role in any of the diet or species effects on the digestibility of these two diets. Higher digestibility of the experimental (higher plant-based protein) diet implies that soybean protein isolate and the other plant protein sources used had a very high digestibility, even

when compared to the meat-based protein sources present in larger proportions in the maintenance diet. On the other hand, the soybean protein sources in the experimental diet may have also been the cause of decreased apparent retention of minerals in both dogs and wolves as compared to the maintenance diet, likely due to the presence of antinutritional factors such as phytate, conglycin, and lectins. Results that differed significantly according to species included dry matter, energy, copper, magnesium, iron, and sodium. In each case, the apparent digestibility or retention were lower in the maned wolves than in the dogs. These findings should alert nutritionists to the possibility of important species differences between maned wolves and other canids in nutrient digestibility, an important consideration since most maned wolves are fed formulas that are, or are based on, domestic canid formulations.

When maintained on both the maintenance and experimental diets for a period of several months, the wolves appeared to do poorly, with gradual weight loss and increasingly poor appetites. In addition, the six maned wolves maintained on these two different diets intended for prevention of cystinuria-related clinical disease exhibited plasma taurine concentrations that were markedly lower than canine and feline normal reference ranges. These deficient concentrations responded within 4 months to taurine supplementation at 0.3% of diet by rising into target normal reference ranges. Further research is needed involving the physiology of taurine metabolism in maned wolves, as the data from this study suggest that taurine metabolism in maned wolves may be different than in other canids. The possibility of a taurine requirement in the maned wolf should be considered. Even if maned wolves

are physiologically capable of taurine biosynthesis, the wolves involved in this study may have been unable to do so due to a lack of substrate (cysteine) availability. A marginal to poor sulfur amino acid status due to urine cysteine loss from cystinuria may have been compounded by the feeding of diets low in cysteine. Several dietary factors may have interfered with normal taurine metabolism in the maned wolves involved in this study, mainly the effects of the types and concentrations of both protein and fiber on nutrient availability, taurine metabolism, and enterohepatic circulation of taurine-conjugated bile salts. The absence of clinical evidence of dilated cardiomyopathy or central retinal degeneration in the taurine deficient maned wolves in this study, in combination with a marked increase in the reproductive success in the US captive population following dietary taurine supplementation, suggest that the clinical consequences of taurine deficiency in this species may be primarily reproductive. In light of our documentation of severe taurine deficiency in all of the maned wolves involved in this study, it should be highly recommended that maned wolves fed diets intended for treatment of prevention of cystinuria-related clinical disease be supplemented with taurine at concentrations according to NRC recommendations for domestic felids.

It is very likely that cystinuria in maned wolves, although not yet scientifically proven, has a genetic origin with a similar molecular basis and pathogenesis to cystinuria in humans and domestic dogs. Previous research as well the first study performed during this thesis demonstrate that nutritional modification, based on principles researched in humans and dogs, may have some benefit in the reduction of

clinical disease secondary to cystinuria. On the other hand, further studies revealed a few important differences between the nutrition of maned wolves and domestic dogs. Therefore, even though domestic canid diets are commonly fed to maned wolves, it is likely that additional significant differences do exist that may affect the nutritional and overall health of this species in captivity. Moreover, differences in nutrient metabolism and utilization in maned wolves emphasizes the necessity of thoroughly researching any diets intended for therapeutic use. The negative aspects of feeding a therapeutic diet that may result in potential nutritional imbalances to wolves unaffected by cystinuria should also be considered. The presence of a renal tubular defect in cysteine reabsorption that is genetically based and present in the majority of both captive and wild populations could be disastrous to the future of this species. The use of nutritional modification to curtail morbidity from this disease is important for the health and quality of life of individual animals, but does nothing to address the underlying primary problem of cystinuria and its high prevalence in the captive population. Although through these studies, as well as previous work, there has been substantial research performed on nutritional management of cystinuria, there has been little performed on other aspects of this disease needed for control and prevention. In the first diet trial, we saw that assessment of cystine crystalluria, as well as the cyanide nitroprusside test, showed promise for use in a qualitative fashion to aid in identification of positive animals. These tests are inexpensive and simple to perform, and thus steps could easily be taken in management of the captive population in order to gain more information regarding the cystinuria status. Successful management of cystinuria would ideally require determination of the

prevalence of the disorder in wild populations, identification and characterization of the molecular and genetic basis of the disorder, the development of a diagnostic test to determine its presence, the identification of carrier animals, and the development of a successful breeding and reintroduction program that does not include affected animals.

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