

# **The Captive Maned Wolf (*Chrysocyon brachyurus*): Nutritional Considerations With Emphasis On Management Of Cystinuria**

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An important and widely recognized condition of the maned wolf is cystinuria, or excess levels of cystine in the urine. Cystinuria has been identified in the majority of maned wolves tested, both in captivity and in the wild. Cystinuria also occurs in humans and domestic dogs, and has been demonstrated in these species to have a genetic basis. In all species afflicted with this condition clinical disease may occur secondary to urolith formation. The solubility of cystine in urine is pH-dependent and the unusually low urine pH of many captive maned wolves may predispose them to morbidity and mortality related to cystine urolithiasis. Dietary modification is a significant component of cystinuria management in both human and canine patients. Based on present knowledge, we hypothesize that through dietary modifications we can reduce urinary cystine concentrations as well as produce alterations in urinary pH which increase cystine solubility and decrease cystine urolith formation in captive maned wolves.

**Key words: urolithiasis; urinary pH; dietary protein; sodium intake**

## **INTRODUCTION**

The maned wolf is a large South American canid that inhabits the grasslands, savannahs, swamplands, and scrub forests of central and eastern Brazil as well as regions of Argentina, Paraguay, Bolivia, and Peru. Due to declining numbers in the wild primarily from habitat destruction and fragmentation, the maned wolf is classified as endangered in its natural habitat by the US Fish and Wildlife Service, as “lower risk, near-threatened” on the 2000 IUCN Red List of Threatened Species, and is listed under Appendix II of the Convention on International Trade in Endangered Species (CITES). A relatively small captive population of maned wolves is maintained in zoological parks

across the United States under the guidance of a maned wolf Species Survival Plan. For much of its history in captivity, the maned wolf has been known to be generally unthrifty and have marginal reproductive success. Relatively poor body condition, extremely rapid passage of digesta, chronically soft stools, and gingivitis are common conditions that are being or have been remedied primarily with husbandry changes [Brady and Ditton, 1978; Bush, 1980]. One serious and widely recognized medical condition afflicting captive maned wolves with an alarming prevalence is cystinuria, or excess levels of the nonessential amino acid cystine in the urine [Bovee and Bush, 1978; Bovee, et al., 1981; Bush and Bovee, 1978; Mussart and Coppo, 1999].

Cystinuria, a disorder which affects both humans and domestic dogs, is an autosomal recessive metabolic defect characterized by defective 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; Tsan et al., 1972]. The disorder is common in humans, with an overall prevalence of approximately 1 in 7,000-15,000, although in certain ethnic groups, such as those of Libyan Jewish descent, the prevalence is as high as 1 in 2500 [Goodyer et al., 2000; Weinberger et al., 1974]. 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; Osborn et al., 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 maned wolves tested [Bovee et al., 1981]. Of the eight wild maned wolves tested, six were found to be positive for cystinuria. Although it was concluded, based on this finding, that the prevalence of cystinuria in wild maned wolves is comparable to that in captive individuals, the extremely low sample size, in addition to the testing of animals from the same geographical region, may have significantly biased the conclusion of a high prevalence in all wild populations. Maned wolves in captivity have historically been plagued by complications related to cystinuria that cause significant morbidity and mortality. To date, no research has been done to characterize the incidence of clinical disease related to cystinuria in wild maned wolves.

Clinical manifestations of cystinuria only develop 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 or lower urinary tract obstruction [Joly et al., 1999; Lindell et al., 1997; Rutchik and Resnick, 1997]. Although cystine and all three of the dibasic amino acids (lysine, ornithine, and arginine) are excreted in excess in cystinuria, only cystine is involved in crystal and urolith formation. Cystine, the least soluble of the amino acids, has a solubility which is highly dependent on pH, with an increase in pH over 7.0

resulting in marked increases in cystine solubility [Barbey et al., 2000; Dent and Senior, 1995; Ng and Streem, 1999].

Amino acids pass freely through the glomerular membrane and their concentration within the glomerular filtrate is equal to that in plasma. Approximately 98% of these amino acids are reabsorbed in the proximal renal tubular epithelial cells and returned to the plasma. Cystine and the dibasic amino acids utilize an identical active tertiary transport mechanism. The high affinity heterodimeric apically-located transporter is composed of heavy (rBAT) and light ( $b^0+$ AT) protein subunits. The gene coding for the rBAT protein, SLC3A1, was localized to human chromosome 2 [Dell and Guay-Woodford, 1999]. More recently, the gene for the  $b^0+$ AT protein subunit was identified on human chromosome 19, and designated SLC7A9 [Goodyer et al., 2000; Palacin et al., 2000]. Although cystinuria was once considered to be a genetically homologous disorder with multiple phenotypic expressions, these findings confirm genetic heterogeneity, with each gene being responsible for one of only two phenotypic categories [Palacin et al., 2000].

Identification of flat, hexagonal cystine crystals in a urine sediment or of cystine calculi by urolith analysis are both pathognomonic for cystinuria, although neither may be present in a cystinuric patient. Elevated urinary cystine levels or a positive cyanide-nitroprusside test may support a diagnosis, but suspicions should ideally be confirmed by direct urine quantification of cystine by HPLC or other methods over a 24-hour period [Rutchik and Resnick, 1997].

Therapy of human and canine cystinuria focuses on increasing the urinary solubility of cystine as well as reducing urinary cystine concentration. These objectives can be accomplished by urinary alkalinization, dietary modification, and administration of thiol-containing drugs [Barbey et al., 2000; Joly et al., 1999; Ng and Streem, 1999]. 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. These and other agents are typically reserved for patients responding suboptimally to other forms of therapy.

Dietary manipulation includes three main factors. Hyperdiuresis decreases urine cystine concentration, decreasing the likelihood of cystine crystallization. Secondly, it has been demonstrated that the amount of cystine excretion is correlated with dietary sodium levels, and thus a low sodium diet is advocated to reduce cystine excretion [Peces et al., 1991]. Finally, a reduction in dietary protein, especially that derived from animal sources which are inherently higher in sulfur containing amino acids, is proposed to decrease urinary cystine excretion as well as increase urinary pH. Alkalinization of the urine to increase cystine solubility can also be accomplished through pharmacological means. Although sodium bicarbonate has been used, potassium citrate has been advocated due to the effects of sodium on cystine excretion.

Although classified in the order Carnivora, the maned wolf's natural diet is not strictly carnivorous. The diet of the maned wolf in the wild consists of approximately 51% plant matter and 49% animal matter [Dietz, 1987; Motta-Junior et al., 1996]. The animal portion consists primarily of small rodents and birds, while the plant portion is largely accounted for by the tomato-like fruit, *Solanum lycocarpum*. In captivity, the maned wolf has traditionally been fed like a carnivore, with dietary protein sources being almost entirely meat based. This was most likely a major contributor to the acidic urine pH (5-6) that is typical of captive maned wolves in the United States, predisposing them to precipitation of cystine and the formation of cystine uroliths [Allen, 2000].

Recent research in this area produced a commercially manufactured maned wolf diet that is currently being fed to all captive maned wolves in the United States [Boniface, 1988]. This diet, as compared to a canine maintenance formula, has higher levels of fat, fiber, and sodium, as well as moderate levels of protein. Most importantly, it contains reduced cysteine and methionine levels, and was demonstrated to result in significantly lower urinary cystine concentrations and improved stool quality in the maned wolves studied.

## Research Goals

The current 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 hypothesize that maned wolves in captivity are predisposed to cystinuria-related complications primarily due to an improper diet that promotes excess cystine excretion and decreased cystine solubility secondary to a low urine pH. By decreasing dietary sodium content and increasing the percentage of dietary protein derived from plant sources we hope, not only to better mimic the nutrient composition of the wild maned wolf diet, but also to decrease urinary cystine concentration and increase cystine solubility through an increase in urine pH. Therefore, dietary manipulations will feature modifications of the current low cysteine diet including a change in the sodium:potassium ratio as well as an increase in the proportion of protein derived from plant sources. Effects of these changes on urine pH, urinary amino acid excretion, and overall digestibility will be monitored. There are three main objectives of this project:

- (1) To develop a new diet for captive maned wolves which results in both a decrease in cystine excretion as well as an increase in urine cystine solubility.
  - a. Decreasing dietary sodium content is proposed to decrease urinary cystine excretion.
  - b. Increasing the proportion of plant-derived protein in the diet is proposed to decrease urinary cystine excretion as well as increase average urine pH.

- c. By increasing urinary pH, the incidence of cystine crystallization and subsequent urolith formation will be decreased.
- (2) To gather baseline digestibility information on the diet on which captive maned wolves in the United States are currently maintained as well as determine effects on digestibility of changes in this diet.
- (3) To lay the groundwork for further studies on the nutrition and physiology of the maned wolf and its successful maintenance in captivity.

## CONCLUSIONS

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. Until such investigations are performed, our efforts will focus on addressing management of cystinuria in captive populations through nutritional modification.

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