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PHOSPHORIC ACID AS A URINARY ACIDIFIER
AND ITS EFFECTS ON SELECTED URINE, PLASMA
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FERRET (MUSTELA PUTORIUS FURO)--A MODEL CARNIVORE

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# PHOSPHORIC ACID AS A URINARY ACIDIFIER AND ITS EFFECTS ON SELECTED URINE, PLASMA AND BONE CHARACTERISTICS OF THE FERRET (MUSTELA PUTORIUS FURO)--A MODEL CARNIVORE

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# ABSTRACT

## PHOSPHORIC ACID AS A URINARY ACIDIFIER AND ITS EFFECTS ON SELECTED URINE, PLASMA AND BONE CHARACTERISTICS OF THE FERRET (MUSTELA PUTORIUS FURO)--A MODEL CARNIVORE

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Three experiments were conducted to explore the use of dietary phosphoric acid as a prophylaxis for struvite urolithiasis and its effects on calcium and phosphorus metabolism in the ferret. Phosphoric acid additions at 1.86 and 2.98% of the dietary dry matter were successful in reducing urinary pH, within four weeks, to a value which would inhibit the formation of struvite uroliths. No signs of metabolic acidosis were observed in the ferrets consuming phosphoric acid at the concentrations provided. High dietary levels of phosphorus resulted in decreased calcium and increased phosphorus concentrations in the urine. Normocalcemia and normophosphatemia were maintained despite the relatively high dietary levels of phosphorus. Dietary phosphoric acid additions and inverse calcium to phosphorus ratios had no detrimental effects on the growth or bone composition of young ferrets.

**TO MY PARENTS, FOR ALLOWING ME TO CHASE MY DREAMS AND HELPING ME TO FIND MY WAY BACK HOME WHEN I GET LOST.**

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Different species of animals have digestive tracts which are adapted to utilize most efficiently the type of food they consume. Herbivores, carnivores, and omnivores are thus classified as such if their diet consists primarily of plants, flesh/meat, or both, respectively.

From a taxonomic viewpoint, the order Carnivora consists of the families Felidae, Viverridae, Hyaenidae, Ursidae, Canidae, Procyonidae, Ailuridedae, and Mustelidae. However, of these families, only the felids (cats), viverrids (civets and genets), hyaenids (hyenas), and perhaps the mustelids (weasels) are "true carnivores". Ursids (bears), procyonids (racoons), and canids (dogs) have omnivorous dietary habits. The ailurids (pandas) are herbivorous (Morris, 1985).

With respect to nutrient requirements, the domestic cat has been the most studied of the carnivores. The nutritional idiosyncracies of the cat support the theory of evolutionary specialization by adherence to a strict carnivorous diet. Cats require a relatively high level of dietary protein because of limited ability to regulate certain catabolic enzymes of amino acid metabolism (MacDonald et al., 1984). The cat does not synthesize niacin from tryptophan, nor convert carotene to vitamin A (Morris, 1982). Cats are unable to convert enough linoleic acid to arachidonic acid, and have a low capacity to synthesize taurine from cysteine (MacDonald et al., 1984). As cats are unable to synthesize

arginine de novo, they are much more sensitive to arginine deficiency than are other mammals, and will exhibit severe signs of ammonia intoxication in less than one hour after consuming a single meal devoid of arginine (Morris and Rogers, 1978a; Morris and Rogers, 1978b). All of the above nutrients that the cat cannot synthesize are found in a carnivorous diet. That is, a cat or other carnivore in its natural environment should never experience any of the mentioned nutritional complications if consuming the carcasses of other animals.

The evolutionary development of the cat has resulted in more stringent nutritional requirements than for omnivores such as the rat, dog and human. What little evidence exists for other carnivorous species, leads to the conclusion that the above mentioned patterns may be common among other strict carnivores (MacDonald et al., 1982).

Another family of carnivores is the Mustelidae, which includes such animals as the marten, otter, mink and ferret. Of the mustelids, only the nutrient requirements of the mink have been firmly established. The domestic ferret, Mustela putorius furo, because of its physiological similarities to both the mink and cat, has been assumed to have nutrient requirements comparable to these species (Bernard et al., 1984).

The domestic ferret originated from the wild European polecat (Mustela putorius) and was first imported into the United States around 1875 (Ryland and Gorham, 1978). The

differences in skull morphology between Mustela putorius furo and the wild Mustela putorius have been ascribed to domestication and hybridization between Mustela putorius and the steppe polecat, Mustela eversmanni (Thornton et al., 1979).

One does not normally think of the ferret as a "common laboratory animal", although it has been used in research for over 50 years. The laboratory ferret first came to prominence with early work on the isolation of canine distemper and human influenza viruses (Bernard et al., 1984). Ferrets are also used for research involving virology, bacteriology, reproduction, teratology, physiology, endocrinology, pharmacology, gastroenterology, embryology, and neurology (Hahn and Webster, 1969; Marshall and Marshall, 1973). The ferret is also considered an excellent animal model for cardiac research (Truex et al., 1974). Ferrets are widely used as a representative carnivorous species for toxicology trials (Bernard et al., 1984). Ferret research publications surveyed from 1977-1983 have established the ferret as a useful experimental laboratory animal (Frederick and Babish, 1983). In excess of 486 research citations were identified, and many more have been published since. Their ease of maintenance, small size, and generally friendly disposition, make ferrets quite suitable for many laboratory studies (Ryland and Gorham, 1978).

The ferret may also be considered a production animal, as it is often farmed for its pelt. There are some discrepancies as to whether the fur marketed as "fitch" is

from the same as or a different animal than the ferret. Gorham (1975) stated that the fur trade calls the skins "fitch" because no woman would want to tell her friends she had a ferret coat! The popularity of raising ferrets strictly for their fur is declining, probably because the fur is coarse and it is difficult to match skins to produce a garment of uniform color and texture (Gorham,1975).

Finally, the ferret is becoming a popular companion animal. However, recent newspaper articles have been giving these animals "bad press". Articles published in various states have called them "mean, vicious little animals" that will "attack infants without provocation" (UPI, 1988). These articles, however, fail to point out the numerous dog and cat attacks which also occur upon infants.

Ferret owners, on the other hand, claim they are quite gentle, with a personality combining the curiousity of a cat with the obedience of a dog. They can be trained to walk on a leash, follow upon command, and be housebroken to a litter box. Even researchers have found the description of the ferret as vicious and dangerous a myth after extensive laboratory experience (Willis and Barrow, 1971). The 1984 estimate of between 20,000 and 30,000 ferrets sold in the United States for pets and laboratory animals (Gorham, 1985) has risen dramatically to more than 5 million pet ferrets in 1988 (Herndon, 1988).

Due to the increasing popularity of ferrets as laboratory and companion animals, research needs to be

conducted in all areas of ferret biology. Studies to determine optimal nutrient levels and of nutritionally related metabolic disorders, are essential to ensure healthy ferret colonies, regardless of their intended usage. Results from these trials will not only benefit the ferret itself, but other species as well, including man.

## Urolithiasis

### A. Background

Urolithiasis is defined as the formation of calculi or an excessive amount of crystals in the urinary tract. These may irritate the mucosal lining, causing cystitis or urethritis; or may become lodged in the urethra or, rarely, the ureters, causing obstruction (Fraser and Mays, 1986). Clinical signs of the disease, occurring singly or in combination, include hematuria, dysuria, and increased frequency of urination with passage of small volumes of urine (Osborne, 1984). In larger domestic animals, signs may include tail swishing, kicking at the belly, repeated twitching of the penis in males, accompanied by strenous efforts to urinate with frequent passage of small amounts of blood-stained urine (Blood, 1982).

The terms urinary calculi, uroliths and stones are used interchangeably to describe organized crystal aggregates found along the urinary tract. More specifically, uroliths are polycrystalline concretions composed primarily of organic or inorganic crystalloids and smaller quantities of organic

matrix (Boyce, 1956; Finlayson, 1974). Most uroliths are composed of a limited number of chemical components including calcium, phosphate, oxalate, uric acid, cystine, silica, magnesium, ammonium, and carbonate. Uroliths are usually classified according to their major component (Bovee, 1982). Uroliths may also be named according to their mineral composition, location in the urinary system, or their shape (Osborne, 1986).

B. Occurrence and composition of calculi

Urolithiasis is recognized in most domestic animals. Livestock and companion animals, as well as wild animals, are afflicted with this renal disorder. Cases of urinary calculi have been reported in cattle (Manning, 1986), sheep (Sutherland, 1985), horses (Caple et al., 1982) and pigs (Djurickovic et al., 1973). The incidence appears to be high in dogs and even higher in cats (Gaskell, 1985). Uroliths, either spontaneous or induced, have also been reported in laboratory animals such as rats (Chow, 1980; Woodard, 1984; Emerick, 1986), mice (DeMars, 1976), monkeys (Resnick, 1978) and rabbits (Sen, 1976). Humans also suffer from urolith formation (Griffith et al., 1981; Brockis et al., 1982).

In the family Mustelidae, many cases of urolithiasis have been documented. It is prevalent in mink and is considered a practical problem on commercial fur ranches (Sompolinsky, 1950; Leoschke et al., 1952). In fact, the mink has been proposed as an animal model for urinary incontinence and urolithiasis (Mitruka, 1976). Some species of otters,

particularly the Asian small-clawed otter, have also been diagnosed with urolithiasis (Nelson, 1983; Karesh, 1984). Urinary calculi are also observed in ferrets, occurring in laboratory research animals as well as ferrets kept as pets (Nyguyen, 1979).

Although urolithiasis is encountered in many species of animals, the composition of uroliths varies from one species to another. A great majority of the uroliths occurring in cattle contain proteinaceous silica. Factors predisposing to silica uroliths include a high silica intake, acid urine and concentrated urine. Also, animals grazing grain stubble (e.g., wheat or sorghum) may experience silica urolith formation since grain stubble has high silica concentration, and grain ingestion can increase urine acidity. Various mixtures of silica and calcium oxalate are also common uroliths in cattle (Manning, 1986).

Sutherland (1958) reported heavy sheep losses in Australia due to urolithiasis over several years when there was an abundance of green herbage. Calcium carbonate was the main component of these uroliths, whereas, cattle grazing on similar pastures had mixtures of silica and calcium oxalate. It is possible that sheep have a more alkaline urine in which calcium carbonate is more likely to precipitate ( Manning, 1986). Calcium, ammonium and magnesium carbonate are also common constituents of calculi in cattle and sheep on pasture. On the other hand, sheep and steers in feedlots usually have calculi composed of calcium, ammonium and

magnesium phosphate, the latter being especially prevalent when sorghum products are a major part of the ration (Blood, 1982).

The predominant form of urolith in the equine is calcium carbonate, probably due to the horse's ability to excrete very high concentrations of calcium in the urine (Caple et al., 1982). The calcium:phosphorus ratio in the urine can also be high, which aids in maintaining calcium homeostasis when dietary calcium is high (Manning, 1986).

Although rare, pigs fed grain and mineral supplements may develop phosphatic uroliths (Manning, 1986). Djurikovic et al. (1973), however, has found urate crystals in the kidneys of piglets.

In the dog, five major types of calculi are seen. The most common type is struvite, composed of magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4.6H_2O$), constituting more than 50% of all reported uroliths (Morris and Doering, 1978). Calcium oxalate is the second most prevalent (7.4-10%), followed by ammonium urate (5.3-7%), silicate (2.4-3.5%) and cystine (2.2-3.2%) (Lewis et al., 1987). Smaller percentages of calcium carbonate (Bovee, 1982) and calcium phosphate (Lewis et al., 1987) calculi have also been reported. Approximately 4.6 -6% of canine uroliths analyzed were of mixed composition (Lewis et al., 1987).

Uroliths of different mineral composition are encountered in a variety of dog breeds. Osborne et al. (1986) most commonly detected uroliths in Miniature Schnauzers, Miniature Poodles, Dachshunds and Shih Tzus. It

appears that, except for Dalmations which commonly form urate stones, small breeds are affected more frequently than large breeds (Brown et al., 1977).

In mature dogs, urolithiasis seems to occur with equal frequency in adults of both sexes. However, about 94% of all cases occurring in dogs less than 1 year old are in males. The mean age of occurrence is 5-8 years (Lewis et al., 1987).

Similarly in cats, the most predominant type of calculus formed is struvite, found in approximately 82% of affected cats (Lewis et al., 1987). The incidence of urinary calculi is high but is sometimes confused with a disease known as Feline Urologic Syndrome (FUS). As the name implies, this disease is a syndrome, multifactorial in origin. FUS constitutes a variety of ailments including cystitis, urethritis, urethral plugs consisting of mucoid proteinaceous material with little or no mineral content, and urolithiasis, with or without urinary obstruction. These conditions, existing alone or in combination, cause hematuria, dysuria, and frequent voiding of small amounts of urine (Lewis, 1981; Bovee, 1982). Currently, the name most in vogue is "lower urinary tract disease of the cat" which better implies a multitude of possible causes (Lewis et al., 1987).

Lewis et al. (1987) report that the majority of clinical FUS cases respond to relief of obstruction, if present, and dietary management that dissolves and prevents formation of struvite microcalculi or crystals in the urinary tract. Therefore, the urinary calculus aspect of FUS may be the most

crucial.

Feline urolithiasis is one of the major health concerns of cat owners today. The disease may occur in either short- or long-haired breeds of cats (Fabricant, 1981). In most cases, the first episode occurs in cats 1 to 3 years old; more than 80% occur in cats 1 to 6 years of age (Lewis et al., 1987). Both male and female cats are subject to urolithiasis. However, male cats with their longer and narrower urethra, are more prone to obstruction than are females (Fabricant, 1981).

Of the mustelids, the uroliths found most commonly in otters, both wild and captive, are composed primarily of calcium salts (Keymer, 1981). Asian small- clawed otters (Aonyx cinerea) have a high incidence of renal and cystic calculi which are mainly calcium oxalate and urate (Karesh, 1983; Nelson, 1983). The reason for the high incidence of renal calculi in Asian small-clawed otters is not known. Several obstructed animals examined did not have hypercalciuria, hyperuricosuria, or hyperoxaluria, which can contribute to calcium oxalate urolithiasis in other species (Calle, 1985).

On the other hand, two closely related members of the Mustelidae family, mink and ferrets, predominantly form struvite urinary calculi. Although there is no doubt that uroliths in mink generally consist of magnesium ammonium phosphate, there have been reported cases of urate calculi (Tomlinson et al., 1987). It is interesting to note that mink and ferrets are prone to develop the same type of

urolith as cats.

Urolithiasis in the farmed mink has been recognized as a major disease concern for over 40 years (Chaddock, 1947). Sompolinsky (1950) documented the occurrence of urolithiasis in mink, as well as in a few weasels and ferrets, raised on fur ranches. In mink, both males and females are susceptible to urinary calculi but at two distinct periods of the year. In the spring, calculi are found primarily in female mink during pregnancy or soon after whelping. In the late summer months, calculi occur mainly in young male kits (Leoschke et al., 1952).

Urolithiasis in mink raised for fur production certainly has economic significance. First, urolithiasis has been suggested as one cause of urinary incontinence and "wet-belly" in mink. This dribbling of the urine may wet, mat and sometimes discolor the fur around the genital orifice. Urinary incontinence may, but does not necessarily, lead to the discoloration of the leather (flesh) side of the pelts in the inguinal region referred to as wet belly (Aulerich et al., 1963a). This disorder, predominantly of male mink, may decrease the value of the pelt by as much as one-third (Aulerich et al., 1963b). Second, the development of calculi in female mink before whelping generally coincides with large numbers of unborn kits (Leoschke et al., 1952). Finally, affected adults may die from uremia if the bladder or urethra is obstructed with calculi, causing hemorrhages (Somplinsky, 1950).

Ferrets are known to develop struvite uroliths, though documented less frequently than those occurring in mink or cats (Bernard et al., 1984). Nguyen (1979) reported urinary calculi in ferrets which were from a laboratory group used for influenza reseach. These were both males and females, ranging from 7 months to 3 years of age. The calculi composed of $MgNH_4PO_4.6H_2O$, were found in the pelvis of the kidney, urinary bladder and urethra. Struvite crystals were also found in the urine of adult, castrated male ferrets, though no urolith formation was detected (Palmore and Bartos, 1987).

C. Calculi formation

Regardless of the composition of or species in which the calculi develop, urolith formation is associated with two complementary but separate phases--initiation and growth. However, initiating events and factors that allow the urolith to grow are not the same for all types of calculi (Osborne and Kruger, 1984).

The initiation phase of urolith formation begins with the formation of a crystal nidus (or crystal embryo), referred to as nucleation (Osborne and Kruger, 1984). The nidus may begin as an aggregate of foreign bodies, bacteria, viruses or cells sloughed from the urinary tract lining (Lewis et al., 1987). This phase is dependent on supersaturation of the urine with calculogenic crystalloids. The urine concentration of urolith-forming constituents may also be influenced by the magnitude of renal excretion of the

crystalloid, time in urinary tract, urine pH, and/or presence of crystal or urolith inhibitors in the urine (Osborne and Kruger, 1984).

Calculi maturation, after formation of the nidus, is a series of processes including crystal growth, epitaxial growth, and/or crystal aggregation (Bovee, 1982a). Crystal growth occurs when the urolith formed is of the same composition as the crystal nidus, provided the urine is highly supersaturated (Osborne and Kruger, 1984). Growth by epitaxy indicates build-up of one type of crystal upon the surface of another type, implying a regular alignment of crystals (Finlayson, 1974). Crystal aggregation refers to the binding of nucleated crystals to one another, leading to the formation of large clusters (Osborne and Kruger, 1984).

It is important to point out that calculi are not just aggregates of crystals, but are comprised of a protein matrix which holds the stones together (Rich, 1968). Urolithiasis is a disease of multifactorial origin, involving factors which promote both crystal and matrix growth. One single, underlying cause cannot be pin-pointed in every instance of urinary calculi formation. Several of the following host and environmental factors have been implicated in the etiology of urolithiasis: intrinsic parameters of the animal such as age, sex, breed, and body weight; season of the year; viral infection; bacterial infection; dietary factors such as water intake, moisture and mineral content, and digestibility of the food; and urine volume and pH. Some of these have been

studied and shown not to be important, while others have a great impact on urolithiasis (Bovee, 1982b).

Struvite uroliths are the predominant stone formed in the ferret, as well as in other carnivores such as the mink and cat. Therefore, the following discussion of possible causes and control of urolithiasis will be aimed primarily toward struvite uroliths. Most of the research has been conducted with the feline, but the conclusions may be applicable to other species which develop struvite calculi, especially those so metabolically similar as the mink and ferret.

D. Contributing factors to urolithiasis

As previously mentioned, there is no breed predilection for urolithiasis in cats. An age-associated risk for urinary calculi peaks at 2 to 6 years of age, and males and females are at equal risk for urolith formation, but males become obstructed more frequently (Willeberg, 1984).

Neutering, especially of the male, was suggested as an increased risk for urolithiasis and urethral obstruction, the hypothesis being that a more narrow urethra would be present if normal development was affected. Bovee (1982) cited no significant difference in the age at which affected cats and normal cats were castrated. Therefore, although castration prior to sexual maturity may affect the normal development of the genitalia, male cats neutered at five months and non-neutered males were not different in urethral diameter (Osborne et al., 1972).

Willeberg (1984) referred to studies which indicated

that excess weight of the cat is associated with urolithiasis. The validity of these claims requires more complete investigation. Confinement of the animal indoors and lack of exercise, both predisposing to obesity, may be secondary factors in urolithiasis.

More realistically, a decrease of physical activity may reduce the frequency of urination, allowing more time for formation of crystals and uroliths (Lewis et al., 1987). Season of the year may have a direct effect on the level of physical activity. That is, during the winter, a cat will be confined more to the indoors. On the other hand, warm weather is more conducive to physical activity (Willeberg and Priester, 1976). Increased physical activity and warmer weather both increase water consumption, and as a result, urine volume. A greater urine volume will decrease the concentration of calculi-forming constituents in the urine and will increase the frequency of urination, both of which reduce the potential for stone formation (Lewis et al., 1987).

Stress has been implicated as a causative factor in numerous diseases of both animals and humans. Caston (1973) suggested that stress is a predisposing cause of urethral obstruction in cats. Also, as previously mentioned, the fact that female mink are more prone to urolithiasis during the spring when whelping occurs, whereas males are more prone during late summer/early fall when "furring" occurs, both stressful periods for the mink, supports the theory of a

"stress factor" in urolithiasis (D. Chausow, personal communication).

Struvite stone formation occurs in alkaline urine and is often considered to be a result of urea-splitting bacterial infections. It is well-recognized that the bacterial enzyme, urease, catalyzes the hydrolysis of urea, thereby increasing urinary osmolarity, alkalinity and local concentrations of ammonia, bicarbonate and carbonate. It is less certain whether this is the only biochemical event involved in the formation of infection stones or whether some other processes may also contribute (Griffith and Osborne, 1987). Organisms such as Staphylococcus, Pseudomonas, and Proteus are able to split urea, thereby inducing alkalinity and oversaturation of urine with respect to $MgNH_4PO_4$ (Nguyen, 1979). The possibility of bacterial infection as a critical etiologic factor in urolithiasis, however, has been ruled out by a number of studies (Reif et al., 1977; Dorn et al., 1973; Schechter, 1970).

Viruses have been implicated in the etiology of urolithiasis. A complex viral etiology, involving a calicivirus and a serologically distinct herpesvirus that produce cellular damage and lead to calculi formation, has been postulated (Bovee, 1982). Rich et al. (1971) were able to isolate a myxovirus from the urinary tract of cats in which obstruction was induced by inoculation with a calicivirus. However, attempts to isolate the virus from naturally obstructed cats or repeat induction of urinary calculi with any virus, or combination of viruses, have

failed (Lewis et al., 1987).

Recently, the role of mycoplasms and urea-plasms in urolith formation in the feline has been examined. These microorganisms, similar to bacteria but without a cell wall, are known to cause urinary infections in humans and other animals (Anonymous, 1988).

The role of diet is certainly one of the more significant factors in urolithiasis. Although it is unlikely to be the sole cause of urolithiasis, there are several ways in which the diet can exert an influence (Burger, 1985). Increased intakes of urolith-forming constituents increase their excretion and concentration in the urine, thus enhancing the formation of uroliths (Lewis et al., 1987).

One major controversy, especially in the cat food industry, concerns wet vs. dry-type diets. With lower food moisture concentrations, there is a different pattern of water balance, with decreased water intake and thus, a lower urine volume and higher urine specific gravity (Gaskell, 1985). Another proposal is that dry foods contain larger amounts of magnesium, a component of struvite, but only when expressed on a caloric basis. That is, when expressed on a dry basis, dry cat foods do not contain more magnesium than other forms of food; instead, the caloric density is less. The cat would need to consume a greater amount of a lower caloric density food to meet its energy requirement, therefore, a greater amount of magnesium is also ultimately

consumed when eating the dry form (Lewis et al., 1987). It is concluded that there is insufficient evidence as to the importance of food moisture, and other researchers maintain that this is not a key factor in urolithiasis (Burger, 1987).

As alluded to above, magnesium content of the diet is suspected to influence urinary calculi formation. Other minerals studied include calcium and phosphorus. Lewis et al. (1978) demonstrated that a diet with a high concentration of magnesium will produce urinary obstruction in cats due to magnesium phosphate calculi. However, this level of magnesium is far in excess of that found in commercial diets, and the calculi formed is not the typical struvite stone seen in the naturally occurring disease.

Dietary calcium and phosphorus levels are also thought to have an influence on urolithiasis, but their precise roles are not completely clear (Burger, 1987). Chow et al. (1975) reported urolith-provoking cat foods contain either low calcium, high phosphate or a calcium:phosphorus ratio less than one. Some researchers recommend a high calcium level to restrict phosphorus excretion and thus inhibit struvite formation. However, Burger (1987) found that a high calcium:phosphorus ratio can <u>increase</u> urine pH which can increase the risk of urolithiasis. In assessing the effect of diet on urolithiasis, it is important to know the mineral composition of the urolith, the species of animal, and the specific minerals involved. Lack of consideration of all these factors has led to much confusion and false information about urolithiasis. One example is the advertising and

confusion concerning the role of the food's ash content in urolithiasis (Lewis et al., 1987).

Ash consists of all non-combustible materials, including all minerals, one of which is magnesium. However, the ash concentration of a food may or may not correlate to its magnesium concentration, and therefore its likelihood of predisposing to or preventing urolithiasis (Lewis et al., 1987). Cat owners, as well as mink farmers, are concerned with the ash concentration of the feed (W. Witz, personal communication).

A diet high in ash because of a high salt concentration may be beneficial in preventing struvite crystallization (Udall and Chow, 1968). A diet high in ash because of high calcium, but low in magnesium, may also be helpful in preventing feline urolithiasis (Lewis et al., 1978). On the other hand, a diet low in ash because of a low calcium concentration, but relatively high in magnesium, is likely to enhance urolith formation (Lewis and Morris, 1984).

The main constituent of urinary calculi in the cat, mink and ferret, namely magnesium ammonium phosphate, is known to form in alkaline urine. Vermeulen et al. (1950) reported a sharp drop in the solubility of $MgNH_4PO_4$ over the range of pH 6.0 to 7.5. In urine, the solubility of struvite increases as the pH decreases below 6.6. The amount of struvite crystals in the urine of healthy cats is directly related to urine pH (Lewis et al., 1987).

That a low urine pH is less likely to result in

precipitation of crystals and, thus, will, reduce the risk of urolithiasis, is currently the focus of attention for many researchers (Burger, 1987). Some researchers (Taton et al., 1984; Cook, 1985) believe urine pH is the most important factor in the prevention and treatment of urolithiasis. In fact, Buffington (1988) reported that if the urine pH is below 6.6, urolithiasis and urinary tract obstruction in cats do not occur--even if the diets are high in magnesium. Normal urine pH values for cats (5.0-7.0)(Kaneko, 1980), mink (6.8-7.5)(Leoschke et al., 1952) and ferrets (6.5-7.5)(Williams, 1976) demonstrate the susceptibility to struvite urolith formation of these animals.

A carnivore's strict adherence to a diet of meat predisposes the animal to excrete an acidic urine. Biochemically, a high-animal-protein diet increases the urinary excretion of calcium, oxalate and uric acid, and reduces urinary pH (Robertson, 1987). The natural production of an acidic urine is due to metabolism of the sulfur-containing amino acids present in the animal tissue (Kane and Douglas, 1987).

Urine pH is not only affected by the diet fed but also by the method of feeding, i.e., meal or free-choice. The phenomenon occurring in all animals after consumption of a meal is called the "post-prandial alkaline tide". Ingestion of food stimulates gastric acid secretion which leaves body fluids more alkaline. To compensate, the kidneys excrete alkaline ions and, thus, raise urine pH (Lewis and Morris, 1984). The greater the amount of food consumed, the more

gastric acid secreted and the higher the urine pH becomes. Thus, when the animal eats all of its food at a single daily meal, the urine pH is elevated more than if the same food is fed ad-libitum, but this increase lasts only a few hours (Lewis et al., 1987). It is not fully elucidated yet which is more important in inducing struvite calculi formation, the magnitude or duration of the pH increase. The answer depends on and varies with the urine pH produced by a particular food (Lewis et al., 1987).

When in the wild, carnivores will usually kill and eat their prey all at once, only every few days, which is similar to the meal feeding pattern. There is some discrepancy as to which feeding method, meal or free-choice, is best for the domesticated small carnivore. One theory suggests that meal feeding, while producing a large alkaline tide for a short period of time, gives an overall urine pH that is lower than if cats are allowed to eat small quantities throughout the day (Burger, 1987). Free-choice feeding may create a continuous alkaline tide, therefore a higher average urine pH. Others believe that eating a number of small meals throughout the day might be advantageous for the production of slightly acid urine, since the post-prandial alkaline tide is lower compared to consumption of a large quantity of food all at once (Kane and Douglass, 1987). If the urine is acidified by manipulation of diet composition, the method of feeding may not be of great importance because the urine pH will always be sufficiently low to prevent precipitation of

struvite uroliths (Burger, 1987).

E. Treatment and Prevention of Urolithiasis

Treatment of urolithiasis is dependent upon the progression of the disease. If urolithiasis has progressed so far that urinary flow is completely obstructed, the animal will die within two to four days if not treated. In some cases, gentle massage may be the only therapy required to remove a calculus if lodged at the external urethral orifice (Lewis et al., 1987). Catheterization with reverse flushing may be required to relieve urethral obstruction (Engle, 1977), and if severe, surgery may be necessary, which entails both high risk for the patient and cost for the client (Hyde, 1987).

If uroliths have not caused obstruction but are present in the urinary tract, removal may also be accomplished by surgical means. A more preferable method is to allow the uroliths to dissolve in the animal by feeding a calculolytic diet, which reduces the concentration of urinary magnesium and results in the maintenance of an acid urine (Lewis et al., 1987). Without proper dietary management, as high as 70% of affected cats will have recurrent urolithiasis (Taton et al., 1984).

"Prevention is the best medicine" is an expression heard often in the clinical area. Urinary acidification may be utilized as a prophylactic measure for urolithiasis. The fact that struvite stones form more readily when urine pH is 7.0 or greater (Lewis et al., 1987), acidifying the urine

increases struvite solubility and decreases crystallization (Taton et al., 1984a).

Feeding a diet that constantly maintains a urine pH of less than 6.4 is not only beneficial in dissolving struvite uroliths, it also helps in preventing the high rate of urolithiasis recurrence (Lewis et al., 1987). Leoschke and Elvehjem (1954) contended that alteration of urinary pH by dietary means is a logical method to attack the urinary calculi disease problem in mink. It follows then that urinary acidification is also a logical method for prevention of urolithiasis in cats and ferrets.

A number of substances have been used in attempts at urinary acidification. Tomato juice and garlic have been advocated, primarily from folklore, as being urinary acidifiers. However, adding one or both to a canned cat food increases the excreted urine pH from 6.7 to 7.1 (Lewis and Morris, 1984). Presently, ethylenediamine dihydrochloride, ascorbic acid, sodium hydrogen phosphate, dl-methionine and ammonium chloride are the most commonly used urine acidifiers.

Bovee et al. (1979) have shown that feeding ethylenediamine dihydrochloride has no effect on the urine pH of the cat. Neither sodium acid phosphate at the dosage level of 3000 mg/day nor ascorbic acid at 1860 mg/day significantly decreased the urine pH of cats (Lewis et al., 1987) and therefore, were presumed ineffective as urinary acidifiers.

Dl-methionine has been administered long-term to cats to prevent recurrence of urolithiasis (Rich et al., 1968;

Engle, 1977). Rich et al. (1968) determined that the dose level of 1.0 g/day of dl-methionine in the ration resulted in a urine pH of around 6.0. The resultant acidification of the urine caused a decrease in the amount of measurable struvite crystals. Unfortunately, methionine has also been shown to be toxic to kittens (Brown and Fox, 1984), and more recently, damaging to adult cat red blood cells at the dosage level of 1.0 g/day (Maede et al., 1987).

Ammonium chloride ($NH_4Cl$) has been effectively used for urine acidification. During metabolism, the ammonia ($NH_3$) is used for protein synthesis or is excreted as urea. The hydrochloric acid (HCl) will acidify body fluids, and the kidneys compensate by excreting an acid urine (Lewis et al., 1987). A diet containing 1.5% $NH_4Cl$ did not lose its effectiveness as a urinary acidifier when fed to cats for 11 months (Taton et al., 1984b). Senior et al. (1986) reported both tablet and powder forms of ammonium chloride were equally effective urine acidifiers in cats fed a commercial cat food. The urine even remained acidic (pH 6.2) during the post-prandial period.

Feeding ammonium chloride to acidify the urine has been practiced in the mink industry since the mid 1950's. Leoschke and Elvehjem (1954) determined that the addition of 1.0 g/mink/day of $NH_4Cl$ is a practical and effective means of preventing the formation of urinary calculi in mink. This regime is a prophylactic measure

since the urinary pH produced by this feeding level of $NH_4Cl$ is not low enough for rapid dissolution of the mink calculi (Leoschke and Elvehjem, 1954).

Ammonium chloride as a urinary acidifier is certainly not without faults. It is not available from most drug or veterinary supply companies or pharmacies (Lewis et al., 1987). Ammonium chloride may also be unpalatable, causing the animals to refuse their feed. For some cats, it may cause vomiting (Lewis et al., 1987). More recently, concern has developed about the long-term acidifying effects of $NH_4Cl$, as well as other urinary acidifiers. Researchers are currently investigating the effects of acidification on blood and bone formation of cats over an extended period of time (Anonymous, 1986).

Another urinary acidifier, phosphoric acid, is recommended for use on fur ranches with mink afflicted with urolithiasis (Witz, 1985). Studies conducted with mink demonstrated the efficacy of phosphoric acid for the prevention of urinary calculi formation. It was found to be advantageous over ammonium chloride for the prophylactic control of urinary calculi. These advantages, reported by Leoschke (1956) are:

(1) More palatable to the mink than $NH_4Cl$,
(2) Definite preservative action on the mink feed (wet type),
(3) May be more effective than $NH_4Cl$ in preventing urinary calculi formation in cases of severe bacterial infection of the urinary tract,and
(4) Phosphoric acid is a natural ingredient of the mink's diet, present as calcium, magnesium, and other mineral salts in horsemeat, fish, and in the bones of animals.

There were no other published data found on the use of phosphoric acid as a urinary acidifier in other animals.

## Calcium, phosphorus and their interrelations

Despite their various important roles, minerals constitute only about 4% of the adult mammalian body (Church and Pond, 1982). Calcium is the most abundant cation, comprising 1 to 2 percent of the total body weight (NRC, 1980). Phosphorus is another very important macromineral. Not only are the absolute levels of both calcium and phosphorus in the body, as well as the diet, critical, but the ratio of calcium to phosphorus is of significance.

Approximately 99 percent of the calcium in the body is found in the bones and the teeth (NRC, 1980). The remaining 1 percent of the body's calcium is widely distributed in the extracellular fluid, soft tissue and various membrane structures (Bronner, 1964). The calcium in the extracellular fluid is critical for muscle contractility, normal neuromuscular excitability, blood coagulation, myocardial function, nerve impulse transmission, and capillary and cell membrane permeability (Simesen, 1980). Calcium also activates numerous enzymes (NRC,1980).

The majority of the body's phosphorus (85%) is also found in the bones and teeth. The remainder is distributed between tissue and membrane components of the skeletal muscle, skin, nervous tissue and other organs (Avioli, 1988). Phosphorus has more known functions than any other mineral

element in the animal body. Phosphorus outside the bone is involved in vital cellular structures and serves in the degradation and synthesis of numerous carbon compounds. When combined with hydrogen and oxygen, phosphorus is capable of forming high-energy phosphate bonds which play a fundamental role in storage, liberation, and transfer of energy in the form of such compounds as ATP, phosphoenolpyruvate and acetyl phosphate (Simesen, 1980). Phosphate may have hormone-like functions in the skeleton since a high serum concentration is associated with increased growth and a low serum phosphate concentration with impaired growth and bone mineralization (Raisz and Kream, 1981). Finally, phosphorus is vitally involved with the buffering of body fluids. The ability of phosphorus to be excreted either as $H_2PO_4^-$ or $HPO_4^{2-}$ gives a broad margin for acid-base metabolism in the body (Simesen, 1980). Phosphate exists in the forms of $HPO_4^{2-}/H_2PO_4^-$ in the glomerular filtrate in a ratio of 4 to 1 which may fluctuate to maintain normal blood pH (Avioli, 1988).

Calcium and phosphate homeostasis is controlled by shifts of calcium and phosphorus among five different compartments: the extracellular fluids; the intracellular pool; the bone and bone fluids; the intestinal lumen; and the renal tubular fluid. Transport between the various compartments is primarily regulated by parathyroid hormone, calcitonin, and cholecalciferol (vitamin $D_3$) (Borle, 1974).

Bone is the only significant storage system for calcium. The calcium concentration of most extraskeletal tissues is

relatively constant throughout life, whereas skeletal calcium concentration varies with age, sex, dietary intake, and physiological state (Bronner, 1982). Physiologic stresses, as well as nutritional deficiencies, influence bone formation and resorption. This exchange process plays a role in calcium homeostasis of body fluids (Menczel et al., 1987).

The mineral phase of bone consists primarily of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. Calcium and phosphorus are closely associated during bone deposition, usually as amorphous calcium phosphate salts which are gradually transformed into hydroxyapatite (Posner, 1973). Borle (1974) states that calcium mobilization from the bone to medium occurs without accompanying phosphate. This would not, however, explain the relative hyperphosphatemia observed in pigs (Miller et al., 1962) and dogs (Jenkins and Phillips, 1960 ) fed calcium-deficient diets. Without adequate dietary calcium, bone resorption occurs to restore plasma calcium levels to normal. The relatively higher inorganic phosphorus serum levels are evidence that calcium and phosphorus are indeed withdrawn together from the bone. Miller et al. (1962) also reported an increase of humoral ash, calcium, phosphorus, and humoral calcium:phosphorus ratio with successive increments of dietary calcium levels from 0% to 1.2%. Perhaps, Borle (1974) was referring to the fluids bathing the bone rather than the plasma when speaking of the "medium".

Calcium and phosphorus occur in an approximate 2:1 ratio in the bone. The ratio considered optimal in the diets of

animals lies within the range of 1:1 and 2:1 (Underwood, 1981). Most animals require a fairly narrow calcium to phosphorus ratio, although ruminants can tolerate wider ratios than monogastric animals, providing the phosphorus level is adequate (NRC, 1980).

Calcium and phosphorus are present in variable amounts in almost all common plant feedstuffs (NRC, 1971). Their levels depend on the plant species, level of soil fertility, and stage of maturity at the time of consumption (NRC, 1980). The pig, cow, and horse can adapt, within limits, to restrictions of supply and increases in demand for calcium and phosphorus (Manston, 1967; Ramberg et al., 1970; Schruyver et al., 1974; Fox and Care, 1978).

But what of the carnivores? Their dietary habit of consuming an all-meat diet is not very conducive to obtaining a dietary calcium to phosphorus ratio between 1:1 and 2:1. For example, McCullough and Ullrey (1983) determined the calcium to phosphorus ratio of white-tailed deer skeletal muscle to be approximately 1:22. This is due to a very low level of calcium (0.028%) with a relatively high phosphorus level (0.612%) in muscle on a dry basis. Horsemeat, which is often fed to felids, has a calcium to phosphorus ratio of 1:10 (Bennet, 1976). Other soft tissues, such as the heart, liver, and viscera, also have inverse calcium to phosphorus ratios (McCullough and Ullrey, 1983).

Unfortunately, misunderstanding of a carnivore's natural dietary habits has led to inappropriate feeding programs in

captivity. As early as 1888, skeletal abnormalities in lions in the London Zoo were noted (Bland Sutton, 1888). Presumably these large felids were fed an all-meat diet without mineral supplementation. It is important to note that when carnivores consume their prey, they may eat the whole body, or significant amounts of mineralized tissue, thus obtaining all necessary dietary minerals (MacDonald et al., 1984). An all-meat diet may result in skeletal abnormalities and induction of a disease referred to as nutritional secondary hyperparathyroidism. This disease is known to occur in both captive wild and domestic carnivores (Fiennes and Graham-Jones, 1960; Krook et al., 1963; Slusher et al., 1965; Palmer, 1968).

Nutritional secondary hyperparathyroidism, also known as osteodystrophia fibrosa, can be summarized as follows: A diet containing excessive phosphorus and insufficient calcium causes hypocalcemia and hyperphosphatemia. Hypocalcemia stimulates the release of parathyroid hormone which causes widespread bone resorption and the development of fibrous connective tissue along with ineffectual osteoblasts in the bones. There is also an increased absorption of dietary calcium from the gastrointestinal tract and an increased excretion of phosphate in the urine (Palmer, 1968). The ultimate loss of bone mass is a result of the resorption of bone produced by parathyroid hormone in order to release calcium, thereby restoring serum calcium levels to normal (Bennet, 1976).

To prevent this disease in captive wild and domestic

carnivores, a commercial nutritionally balanced diet should be substituted for a meat diet (Bennet, 1976). If the diet is "home-made" and the primary ingredient is muscle meat and/or other soft tissue, calcium supplementation may be necessary. Another solution for the feeding of large captive wild carnivores is to allow the animal access to a whole carcass (R. Barbiers, personal communication). This permits the animal to eat a more naturally well-balanced meal since it is allowed not only to consume muscle, but also skin, cartilage, and bones. These portions assist in meeting the nutritional requirements, and are also beneficial for proper oral hygiene (Vosburgh et al., 1982; Haberstroh et al., 1984).

Many other bone disorders are concerned with the calcium and phosphorus levels in the body. Rickets, a disease associated with growing animals, is a failure of calcium salts to be deposited in the bone matrix. The cause may be an inadequate supply of calcium and/or phosphorus and/or vitamin D. In the mature animal, a similar lack of these nutrients results in osteomalacia which is manifested by reabsorption of bone already laid down (Simesen, 1980).

Osteoporosis is a disease in which the formation of the osteoid matrix is abnormal. There is a net resorption of bone tissue which is not associated with disturbances in calcium and phosphorus metabolism (Simesen, 1980). That is, there is a decrease in bone mass while the ratio of minerals to organic matrix appear normal. Although the precise

etiology of osteoporosis has not yet been elucidated, early studies suggested that dietary calcium deficiency may be an important factor. However, dietary histories in normal and osteoporotic populations have failed to substantiate this suggestion (Jowsey, 1975).

High dietary protein has been implicated as a possible contributory factor in the etiology of osteoporosis in humans due to increased calcium excreted in the urine and a negative calcium balance (Johnson et al., 1970; Walker and Linkswiler, 1972). In these studies, however, the diets also supplied high levels of phosphorus. Kim and Linkswiler (1979) found that hypercalciuria and a negative calcium balance induced by incorporating casein, lactalbumin and wheat gluten into the diets of men, were accompanied by a 23% increase in urinary hydroxyproline, indicating an increase in bone resorption. This suggests that the protein-induced calcium loss is the result of loss of bone mass and, consequently, of calcium . Urinary calcium was found to double (182 to 380 mg/day) with an increased protein intake from 50 to 150 g in men (Zemel et al., 1981), probably due to an increased glomerular filtration rate and a decreased fractional renal tubular reabsorption of calcium.

Human subjects fed a high-protein diet, given as purified proteins, have demonstrated increased calcium loss via the urine (Linkswiler et al., 1974), whereas dogs did not (L. Case, unpublished data). Increased urinary calcium excretion was not observed in humans, however, when the high-protein diet was a natural, complex protein, namely meat

(Spencer et al., 1978b; Spencer et al, 1983). In an animal study in which beef was used as the protein source, there was no adverse effect on bone with high protein intake (Calvo et al., 1982). The different results obtained with purified and complex dietary proteins may be due to the high phosphorus content of red meat (Spencer et al., 1988). Similarly, a high protein intake from milk does not increase urinary calcium and in fact, a decrease was observed, apparently due to the high phosphorus content of milk (Spencer et al., 1984).

A high phosphorus intake has been shown to result in a decrease in urinary calcium in man (Goldsmith et al., 1976; Bell et al., 1977; Spencer et al., 1978a). Anderson and Draper (1972) postulated that the decrease in urinary calcium during high phosphorus intake is due to a renal effect of phosphate. In addition, Spencer and co-workers (1978a) have utilized orally administered tracer doses of $^{47}Ca$ to show that the decrease in urinary calcium during high phosphorus intake is not a result of decreased intestinal absorption of calcium. The decrease in urinary calcium may be due to a decrease in bone resorption or to an increase of bone formation and mineralization (Spencer et al., 1978b). Phosphate acts directly on osteoblasts to increase bone formation and mineralization (Feinblatt et al., 1970), and also increases collagen synthesis <u>in vitro</u> (Flanagan and Nichols, 1969). On the other hand, much research has shown that high dietary phosphorus decreases calcium and phosphorus

concentrations in bones of aged mice and rats (Anderson and Draper, 1972; Draper et al., 1972; Krisnarao and Draper, 1972). In addition, Sie et al. (1974) have reported that high dietary phosphorus increases phosphorus and decreases calcium in the serum of rats. It is postulated that when serum calcium is decreased, parathyroid hormone secretion is stimulated, which in turn, increases bone resorption (Sie et al., 1974)

The effects of phosphorus in humans discussed above apply to dietary phosphorus and not to phosphate contained in carbonated soft drinks (Spencer et al., 1988). In young adults, the consumption of soft drinks led to a decrease in ionized serum calcium, an increase of the serum parathyroid hormone level, and an increase in urinary hydroxyproline and cyclic AMP, changes that have been interpreted as a result of increased parathyroid function (Bell et al., 1977). Most carbonated soft drinks contain phosphoric acid, as well as citric acid, which may adversely affect bone due to induction of acidosis (Spencer et al., 1988).

Phosphate stored in the bone serves not only as a primary source of chemical energy for metabolic use, but also as a buffer in blood and urine. Wachman and Bernstein (1968) suggested that ingestion of acid-ash diets may contribute to osteoporosis by life-long utilization of the buffering capacity of basic salts in bone to maintain pH homeostasis. Metabolic acidosis produces increased urinary calcium excretion by causing decreased renal tubular calcium reabsorption (Lemann et al., 1967). Early studies have shown

that urinary calcium excretion is increased during acidosis induced by the feeding of hydrochloric acid (Goto,1918) or ammonium chloride (Farquharson et al., 1930). Endogenous sulfate production, whether from high protein feeding (Whiting and Draper, 1980) or supplementation with sulfur-containing amino acids (Whiting and Draper, 1980; Zemel et al., 1981), is associated with an increase in urinary calcium excretion. Whiting and Draper (1980) suggested that protein-induced hypercalciuria results from a decrease in renal calcium reabsorption due to increased endogenous sulfuric acid production. Acid loading with ammonium chloride or acid phosphate has been reported to cause calcium loss via urine and feces with a resultant lowered bone density in the growing rat (Petito and Evans, 1984). In addition, high acidity diets have been reported to increase bone resorption, which may lead to skeletal ostopenia (Milligan and Evans, 1978).

Although many factors may contribute to osteoporosis, such as hormones, drugs and alcohol (Spencer and Kramer, 1986), complicated by race, genetics and occupational influences (Draper and Scythes, 1981), the role of nutrition is critical. There exists a very complex metabolic interaction among the type and level of protein consumed, phosphorus intake, diet acidity, and calcium and phosphorus metabolism in the body. The calciuric effect of high protein diets is apparently due primarily to increased acid production, as sulfate, arising from the oxidation of excess

sulfur amino acids (Whiting and Draper, 1980). Hypercalciuria is therefore more severe when proteins high in sulfur amino acids are consumed. Phosphorus supplementation effectively prevented the hypercalciuria caused by the addition of sulfur amino acids to a low protein diet (Zemel et al., 1981).

Because increased protein and phosphorus intakes have opposite effects on calcium metabolism, a simultaneous increase in both is likely to have a much less dramatic effect than an increase in one or the other alone (Schuette and Linkswiler, 1982). Hegsted et al. (1981) demonstrated that the level of dietary phosphorus modifies the effect of an increase in dietary protein on urinary calcium and calcium balance. That a high protein intake which is due to a high meat diet, even though it is an acid-ash diet, does not lead to a significant loss of calcium and that urinary calcium decreases with time in those cases in whom this increase occurred (Spencer et al., 1978b), is an example of this peculiar relationship between dietary phosphorus, protein, and acid-base balance.

Further work is necessary to elucidate the complex interactions among dietary protein, phosphorus and calcium. The net effect of high protein diets, as normally consumed, on bone metabolism is not yet completely understood (Draper and Scythes, 1981).

## EXPERIMENTS 1 AND 2

Urolithiasis is a renal disease defined as the formation of calculi or an excessive amount of crystals in the urinary tract which may result in hematuria, dysuria and increased frequency of urination with passage of small volumes of urine (Osborne, 1984). Representative carnivores, the cat and mink, both have a tendency to form struvite (magnesium ammonium phosphate) uroliths (Lewis et al., 1987; Sompolinsky, 1950). Ferrets also have been reported to be afflicted with struvite uroliths (Nguyen, 1979).

Many factors have been proposed as the cause of urolithiasis, such as bacterial infections (Griffith and Osborne, 1987), viral infections (Rich et al., 1971), stress (Caston, 1973), inappropriate diet (Chow et al., 1975; Lewis et al., 1978), and high urinary pH (Taton et al., 1984a; Cook et al., 1985). That a low urine pH is less likely to result in precipitation of struvite crystals in the urine and, thus, will reduce the risk of urolithiasis is currently the focus of attention for many researchers (Burger, 1987).

Urinary acidification has been used for both treatment (Lewis et al., 1987) and prevention (Leoschke and Elvehjem, 1954; Taton et al., 1984a) of struvite urolithiasis in animals. Substances used to acidify the urine of animals include ethylenediamine dihydrochloride, ascorbic acid, sodium hydrogen phosphate, dl-methionine and ammonium chloride. Ethylenediamine dihydrochloride, ascorbic acid

and sodium hydrogen phosphate all have been shown to be fairly ineffective as urinary acidifiers for cats (Bovee et al., 1979; Lewis et al., 1987). Dl-methionine has been reported to acidify the urine of cats and to decrease the amount of measurable struvite crystals (Rich et al., 1968). However, methionine also has been shown to be toxic to kittens (Brown and Fos, 1984) and damaging to adult cat red blood cells (Maede et al., 1987). Ammonium chloride has been effectively used as a urinary acidifier for the cat (Senior et al., 1986) and mink (Leoschke and Elvehjem, 1954). Unfortunately, ammonium chloride may cause feed refusal and vomiting (Lewis et al., 1987).

Phosphoric acid ($H_3PO_4$) is another substance which is recommended for use on fur ranches with mink afflicted with urolithiasis (Witz, 1985). Leoschke (1956) determined that 0.6% of 75% $H_3PO_4$, added to a wet diet, was safe and effective through the growth and reproductive phases of the mink. Data from research with other domestic carnivores, concerning the effects of $H_3PO_4$ as a urinary acidifier, have not been found.

Calcium (Ca) and phosphorus (P) metabolism in the body is very closely integrated, and many factors influence their status. A dietary imbalance of one may affect the body's utilization of the other, often adversely. For example, a dietary Ca deficiency may result in hyperphosphatemia (Miller et al., 1962) and a diet high in P may result in hypocalciuria (Goldsmith et al., 1976).

Metabolic acidosis has been reported to cause increased

excretion of both Ca and P in the urine (Lemann et al., 1967; Irving, 1969). Protein-induced hypercalciuria has also been observed in human subjects (Kim and Linkswiler, 1979; Zemel et al., 1981). On the other hand, Spencer and co-workers (1983) did not observe any increase in urinary calcium when humans were fed a high protein diet supplied as meat.

Calcium and P levels in the diet are also thought to have an influence on the development of struvite urolithiasis, but their precise roles are not completely clear (Burger, 1987). Chow et al. (1975) reported that cat foods containing either low Ca, high phosphate, or a Ca to P ratio less than one, were urolith provoking. Conversely, Burger (1987) found that a high Ca to P ratio can raise urinary pH which may increase the risk of urolithiasis.

The question, therefore, arises as to which factor takes precedence in a diet containing high P, acid-ash and high protein? Also, what are the effects of dietary Ca and P concentrations and ratios in relation to struvite urolithiasis? To explore these questions, the ferret was used as a model carnivore in two experiments examining the efficacy of $H_3PO_4$ as a urinary acidifier. Other factors of interest that were examined included the effects of $H_3PO_4$ on urine Ca and P concentrations, as well as on plasma Ca, inorganic P and alkaline phosphatase.

Experiments were approved by the All University Committee on Animal Use and Care (AUCAUC) of MSU.

## Experiment 1

### Materials and Methods

**A. Animals and procedures**

Forty-eight adult ferrets, 24 males and 24 females, were obtained from Pheonix Farms, Marcellus, Michigan, for use in this preliminary study of the effects of phosphoric acid ($H_3PO_4$) added to the diet as a urinary acidifier and as a source of phosphorus (P). Four males and four females were randomly assigned to each one of the six test diets. All animals were housed indoors, individually, in galvanized steel welded wire cages at the Michigan State University Fur Farm Facility. The cages measured 61(W) x 76(L) x 46(H) cm and were suspended 61 cm above the floor. The lighting was controlled to simulate natural outdoor conditions. Food and water were provided ad libitum, fresh daily, for a 28-d trial period.

On a weekly basis, ferrets were weighed and their feed consumption measured. Average daily feed intake was measured by providing the ferrets a weighed amount of food, in excess of what they would consume, and subtracting the orts on the following day for four consecutive days.

At 2-wk intervals, ferrets were placed in individual metabolism cages for the purpose of urine collection. Mid-morning, immediately after urine was voided, its pH was measured with a glass electrode pH meter. Urine samples were then stored at -20C until further analyses could be performed.

Table 1. Composition of Ferret Diets (Experiment 1)

| | TREATMENT | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Ingredient | % of dietary DM | | | | | |
| Ground beef | 63.4 | 63.4 | 63.4 | 63.4 | 63.4 | 63.4 |
| Cereal by-product[a] | 27.0 | 27.0 | 27.0 | 27.0 | 27.0 | 27.0 |
| Soybean meal[b] | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Calcium carbonate | .69 | .69 | 1.49 | 1.49 | 3.07 | 3.07 |
| Phosphoric acid (85%) | .48 | 2.71 | .48 | 2.71 | .48 | 2.71 |
| Alpha-cellulose[c] | 4.83 | 2.60 | 4.03 | 1.80 | 2.45 | .22 |
| MSU VTM premix[d] | .50 | .50 | .50 | .50 | .50 | .50 |
| Vitamin E premix[e] | .05 | .05 | .05 | .05 | .05 | .05 |
| Selenium premix[f] | .05 | .05 | .05 | .05 | .05 | .05 |
| % Ca | .3 | .3 | .6 | .6 | 1.2 | 1.2 |
| % P | .6 | 1.2 | .6 | 1.2 | .6 | 1.2 |
| Ca:P | 1:2 | 1:4 | 1:1 | 1:2 | 2:1 | 1:1 |

[a]Kel-Mix, Kellogg Co., Battle Creek, MI.
[b]44% crude protein.
[c]Solka-Floc, Brown Co., Boston, MA.
[d]Vitamin-trace mineral premix. Vitamin concentrations (per kg): retinyl acetate, 660,000 USP units; cholecalciferol, 132,000 USP units; riboflavin, 660 mg; d-calcium pantothenate, 2,640 mg; nicotinic acid, 3,520 mg; cyanocobalamin, 3.96 mg; choline chloride, 25,344 mg; menadione sodium bisulfite complex, 440 mg. Mineral concentrations in % (and source): zinc, 1.496 (zinc oxide); iron, 1.188 (ferrous sulfate); manganese, .748 (manganous oxide); copper, .198 (copper oxide); iodine, .06 (potassium iodate).
[e]500,000 USP units supplied per kg.
[f]200 mg Se supplied per kg.

At the conclusion of the 4-wk trial, ferrets were anesthetized with xylazine[1] (.3-1 mg/kg body weight) and ketamine hydrochloride[2] (11-15 mg/kg body weight) administered intramuscularly. Blood samples (10 ml) were obtained from each ferret via cardiac puncture as described by Wechsler (1983), using 18 gauge, 3.8 cm needles with heparinized syringes. The heparinized blood was centrifuged at 2100 x g for 15 min. Plasma was harvested and stored in plastic tubes at -20C until further analyses were performed.

B. Diet composition

A 2 x 3 factorial design was developed by additions of phosphoric acid ($H_3PO_4$) and calcium carbonate ($CaCO_3$) to a meat- and cereal based diet. The diets were formulated to contain P at either .6 or 1.2% of the dry matter, and Ca at .3, .6 or 1.2% of the dry matter (Table 1). The resulting Ca:P ratios were 1:1, 1:2, 1:4 or 2:1.

C. Laboratory analyses

Urine

To a 5-ml sample of thawed urine, 5 ml of nitric acid and 1 ml of perchloric acid were added in a 50-ml Erlenmeyer flask. Samples were heated on a hot plate until the acid digestion was complete. Distilled deionized $H_2O$ was added to bring the volume to 50 ml. To a .3-ml sample of this diluted digest was added 5.7 ml of 10,000 ppm of $SrCl_2$ for calcium analysis by atomic absorption spectrophotometry

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[1]Rompun, Miles Laboratories Inc., Shawnee, KS.
[2]Vetalar, Parke-Davis, Morris Plains, NJ.

(Instrumentation Laboratories Model 951, Instrumentation Laboratory Inc., Wilmington, MA). A .1-ml . volume of the digested, diluted urine was further diluted with .9 ml of $H_2O$ for P analysis by the colorimetric method of Fiske and Subbarow (1925).

Plasma

Four ml of 12.5% trichloroacetic acid were added to 1-ml plasma samples and centrifuged for 15 min at 900 x g. The de-proteinized supernatant was transferred to plastic tubes for storage. A 1-ml sample of the supernatant was mixed with 1 ml of 20,000 ppm $SrCl_2$ and analyzed for Ca concentration by atomic absorption spectrophotometry. The supernatant was also used for P determination by the Fiske and Subbarow method (1925). Alkaline phosphatase (ALP) activities were measured in the whole plasma by Sigma procedure #245 (Sigma Diagnostics, St. Louis, MO).

D. Statistical analyses

All data were subjected to analysis of variance by the SAS general linear model procedures (SAS Institute Inc., Cary, NC).

## Results and Discussion

Bleavins and Aulerich (1981) reported daily feed consumption for ferrets to be in the range of 39 to 80g, on a dry matter basis, depending on sex. Mean values for feed intake (g as fed/d) of males and females consuming the test

diets are presented in Table 2. When converted to a dry matter basis, these values were similar to those reported above. Diets providing Ca:P ratios of 1:2 (Treatments 1 and 4) resulted in the greatest average daily feed intake (73 g dry matter/d). Only feed consumption on an as fed basis was analyzed statistically. High dietary concentrations of both Ca and P (Treatment 6) resulted in the lowest average daily feed intake of 53 g/d.

Treatment group 1 not only had the greatest average daily feed intake, but also exhibited the greatest negative body weight change after 4 wk on experiment (Table 3). Ferrets consuming 1.2% dietary P all maintained or gained weight. Ferrets consuming high dietary Ca demonstrated negative weight changes as a percent of the initial weight. Based on these results, 1.2% dietary P appears to improve feed efficiency vs .6% dietary P when dietary Ca is at least .6%.

There was a significant ($P < .01$) interaction between dietary Ca and P on urinary pH. Figure 1 depicts the effects of dietary P concentrations at various Ca concentrations on urine pH. Dietary levels of 1.2% P resulted in lower ($P < .01$) urinary pH only at the 1.2% dietary Ca level. Overall, ferrets consuming Treatment 2 with a Ca to P ratio of 1:4 had the lowest mean urine pH. Although the urine pH of all groups decreased between the 2- and 4-wk sampling times, the period effect was not significant.

There was also a significant ($P < .01$) Ca x P interaction

Table 2. Average Daily Feed Intake of Ferrets[a] (Experiment 1)

| Treatment | %Ca | %P | Ca:P | Feed intake (g/d) as fed | Feed intake (g/d) dry wt |
|---|---|---|---|---|---|
| 1 | .3 | .6 | 1:2 | 158 | 78.5 |
| 2 | .3 | 1.2 | 1:4 | 124 | 59.6 |
| 3 | .6 | .6 | 1:1 | 115 | 54.6 |
| 4 | .6 | 1.2 | 1:2 | 142 | 68.2 |
| 5 | 1.2 | .6 | 2:1 | 117 | 56.7 |
| 6 | 1.2 | 1.2 | 1:1 | 109 | 53.1 |

[a]Eight observations per mean. Twenty-eight day trial.

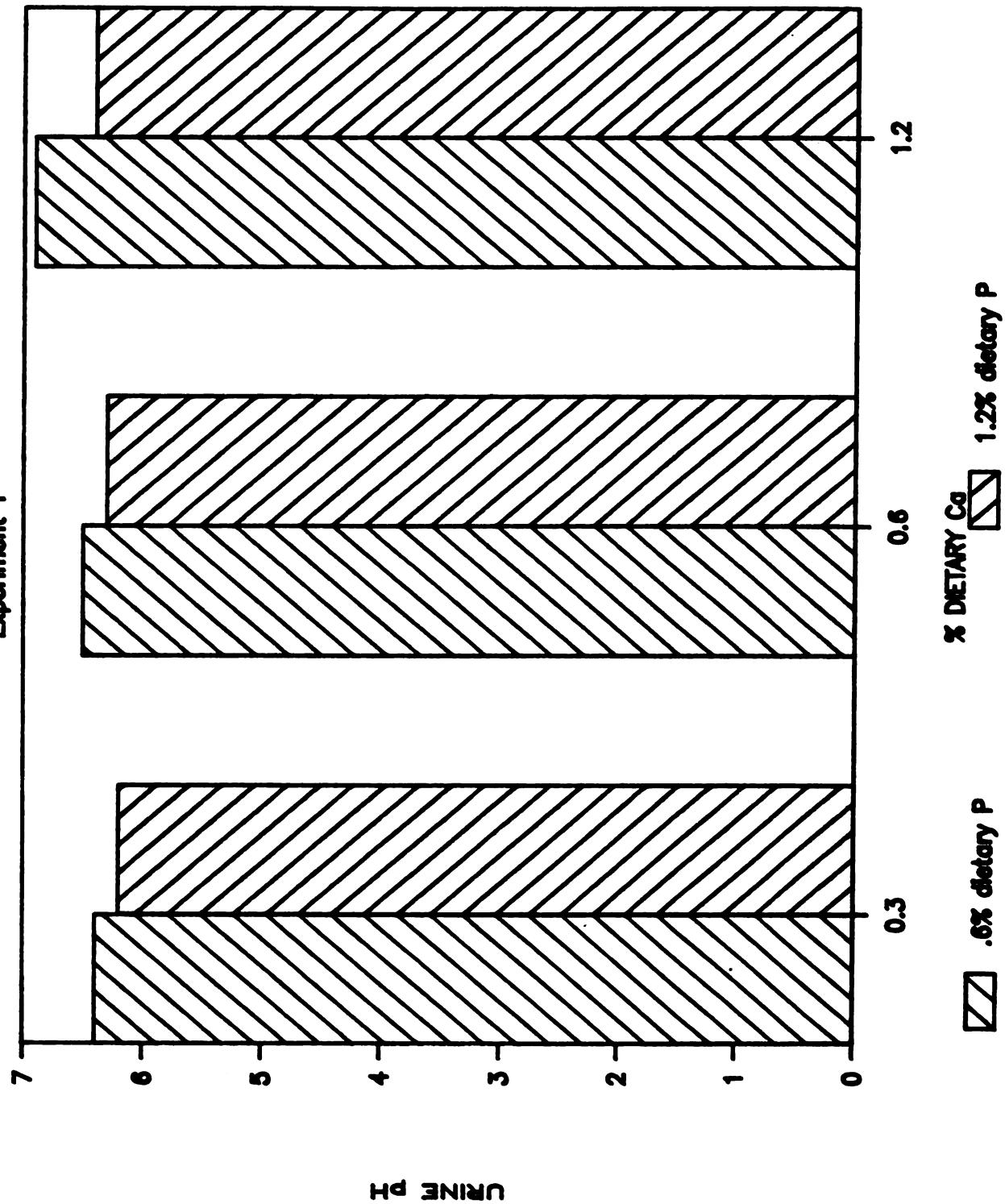
Table 3. Body Weight Changes of Ferrets[a] (Experiment 1)

| Treatment | %Ca | %P | Ca:P | Weight change (% of initial) |
|---|---|---|---|---|
| 1 | .3 | .6 | 1:2 | (-) 4.65 |
| 2 | .3 | 1.2 | 1:4 | (-) .07 |
| 3 | .6 | .6 | 1:1 | (-) 1.42 |
| 4 | .6 | 1.2 | 1:2 | (+) 1.86 |
| 5 | 1.2 | .6 | 2:1 | (-) 5.58 |
| 6 | 1.2 | 1.2 | 1:1 | (+) 3.20 |

[a]Eight observations per mean. Twenty-eight day trial.

**Figure 1.** **The effects of dietary calcium and phosphorus concentrations on urine pH of adult ferrets. SEM and significant differences are presented in Table 6 (Experiment 1).**

FIGURE 1. URINE pH



on urine Ca concentration. Treatment 5 with a Ca:P ratio of 2:1 produced a higher urinary Ca concentration than the other treatments (Figure 2). The relatively high urine Ca concentration (6.1 mg/dl) resulting from Treatment 5, as compared with Treatment 6, was presumed to be the result of the high dietary Ca (1.2%) provided and the relatively low level of P (.6%). As suggested by Spencer et al. (1978b), the decrease in urine Ca in Treatment 6 may be due to increased bone mineralization at the high level of P intake. Within each level of dietary Ca, P supplied at 1.2% of the diet resulted in a lower urinary Ca concentration than when P was supplied at .6%, and this difference was significant ($P < .01$) when the diet contained 1.2% Ca.

There was no significant Ca x P interaction on urine P concentration. There were, however, significant ($P < .01$) independent effects of dietary Ca and P. Treatment groups consuming the highest level of Ca (1.2%) had lower ($P < .05$) urine P concentrations than those consuming .3% or .6% Ca (Table 4). In addition, those treatment groups consuming 1.2% dietary P exhibited a higher ($P < .001$) urine P concentration than those consuming .6% dietary P (Table 5). Figure 3 illustrates the mean urine P concentrations found in each dietary treatment.

Dietary Ca and P levels also had independent effects on plasma Ca concentrations. Diets containing 1.2% Ca (Treatments 5 and 6) produced a greater ($P < .01$) mean plasma Ca concentration than diets containing either .3% or .6% Ca (Table 4). Phosphorus in the diet at 1.2% also produced

**Figure 2.** The effects of dietary calcium and phosphorus concentrations on urine calcium concentrations of adult ferrets. SEM and significant differences are presented in Table 6 (Experiment 1).

# FIGURE 2. URINE Ca CONCENTRATION

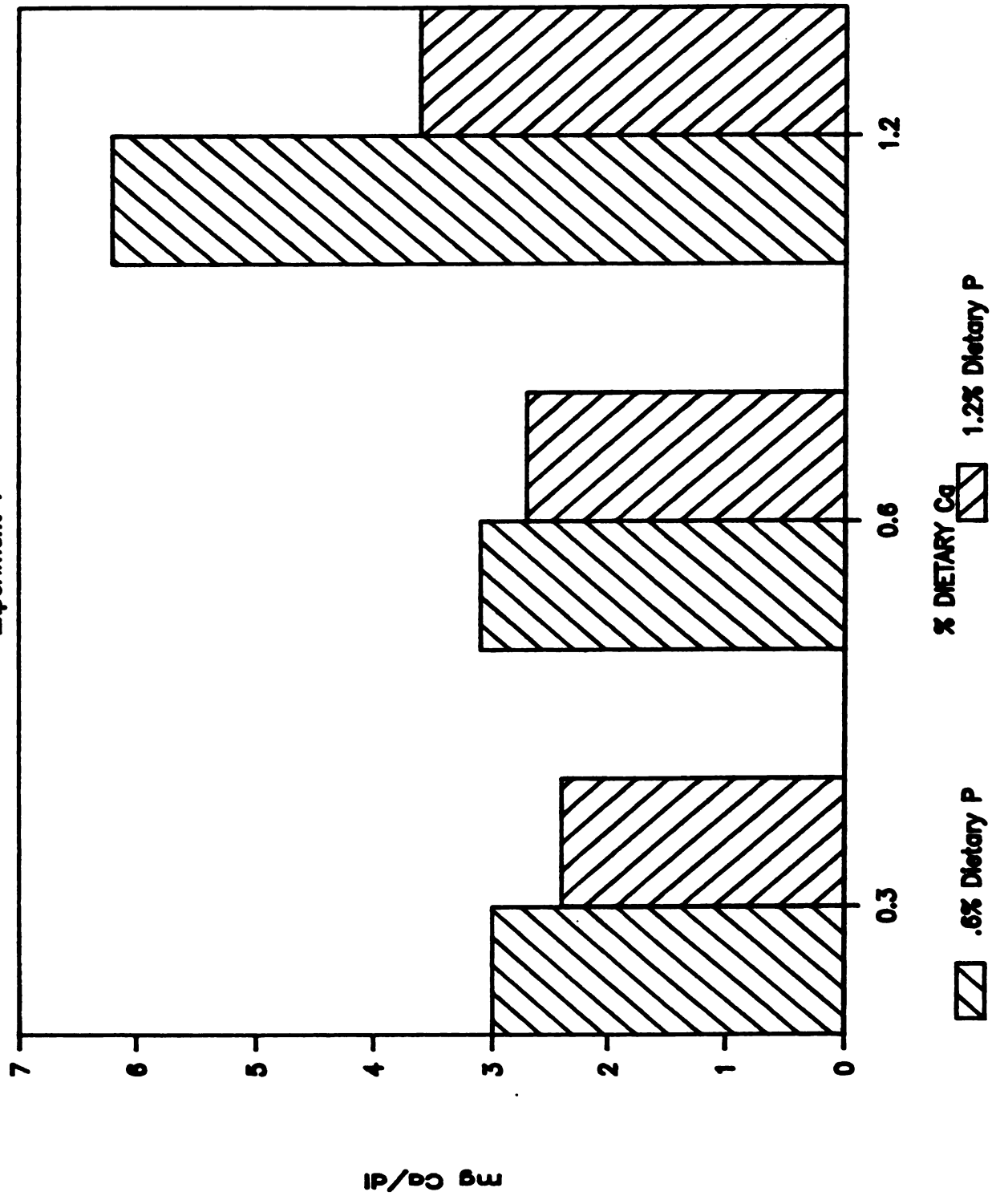


Table 4. Effects of Various Levels of Calcium in the Diets of Ferrets on Selected Urine and Plasma Values[a] (Experiment 1)

| | Dietary Ca (% of dry matter) | | | | |
|---|---|---|---|---|---|
| Measure | .3 | .6 | 1.2 | SEM | P< |
| Urine pH | 6.28 | 6.38 | 6.62 | .054 | .001[c] |
| Urine Ca (mg/dl) | 2.8 | 3.0 | 4.9 | .34 | .001[c] |
| Urine P (mg/dl) | 230[d] | 227[d] | 167[e] | 15.7 | .001 |
| Plasma Ca (mg/dl) | 9.8[f] | 10.0[f] | 10.3[g] | .10 | .01 |
| Plasma inorg. P (mg/dl) | 6.8 | 5.9 | 5.7 | .17 | .001[c] |
| Plasma ALP (U/L)[b] | 20.5 | 20.2 | 18.9 | 1.14 | NS |

[a]Sixteen observations per mean.
[b]One unit of activity defined as that amount of enzyme which will produce 1 micromole of p-nitrophenol per min under conditions of Sigma assay procedure #245.
[c]Significant (P <.01) Ca x P interaction.
[de]Means within a row with different letter superscripts are significantly different (P <.05).
[fg]Means within a row with different letter superscripts are significantly different (P <.01).

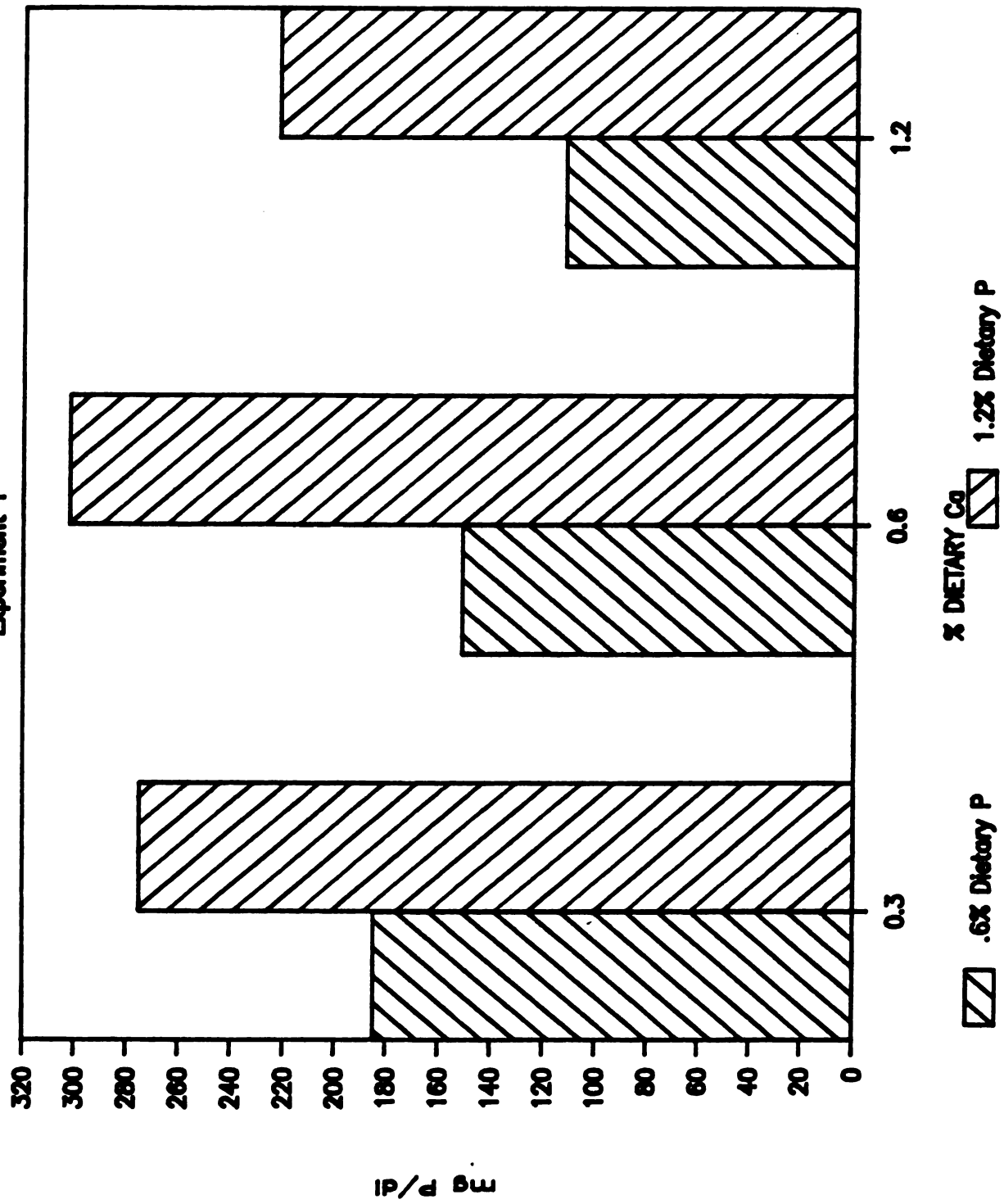
Table 5. Effects of Various Levels of Phosphorus in the Diets of Ferrets on Selected Urine and Plasma Values[a] (Experiment 1)

| Measure | Dietary P (% of dry matter) .6 | 1.2 | SEM | P< |
|---|---|---|---|---|
| Urine pH | 6.58 | 6.28 | .044 | .001[c] |
| Urine Ca (mg/dl) | 4.1 | 2.9 | .28 | .001[c] |
| Urine P (mg/dl) | 150 | 266 | 1.28 | .001 |
| Plasma Ca (mg/dl) | 9.8 | 10.2 | .08 | .001 |
| Plasma inorg. P (mg/dl) | 5.9 | 6.4 | .14 | .05[c] |
| Plasma ALP (U/L)[b] | 18.4 | 21.3 | .93 | .05 |

[a]Twenty-four observations per mean.
[b]One unit of activity defined as that amount of enzyme which will produce 1 micromole of p-nitrophenol per min under the conditions of Sigma assay procedure #245.
[c]Significant (P <.01) Ca x P interaction.

**Figure 3.** **The effects of dietary calcium and phosphorus concentrations on urine phosphorus concentrations of adult ferrets. SEM and significant differences are presented in Tables 4 and 5 (Experiment 1).**

# FIGURE 3. URINE P CONCENTRATION



greater (P <.001) plasma Ca concentrations than diets with .6% P (Table 5). This contradicts results reported by Miller and co-workers (1964) who observed a decrease in serum Ca in baby pigs with increasing dietary P.

There was a significant (P <.001) dietary Ca x P interaction upon plasma inorganic P concentrations (Table 6). Treatment 2, with a 1:4 Ca:P ratio, resulted in higher (P <.01) plasma inorganic P concentrations than any of the other diets. Treatment 4, with a 1:2 Ca:P ratio, also produced higher (P <.05) plasma inorganic P concentrations than either Treatments 5 (Ca:P of 2:1) or 6 (Ca:P of 1:1).

Dietary Ca concentrations had no significant effect on plasma ALP activity, nor was there a significant dietary Ca x P interaction. Dietary P concentrations, however, did have a significant (P <.05) effect (Table 5). Ferrets consuming 1.2% P in the dietary dry matter had a higher (P <.05) mean ALP activity than those consuming .6% P. Although this difference was statistically significant, the relative increase from 18.4 to 21.3 U/L is probably not physiologically significant, considering the normal range for ferrets is 11 to 84 U/L (Thornton et al., 1979).

This preliminary study provided some insight into the ferret's ability to maintain Ca and P homeostasis when faced with dietary challenges in concentrations of these elements. Inverse dietary Ca:P ratios appear to be beneficial for reducing urinary pH of the ferret.

Due to the effects of a Ca x P interaction on many of

Table 6. Effects of Various Calcium to Phosphorus Ratios in the Diets of Ferrets on Selected Urine and Plasma Values[a] (Experiment 1)

| | Dietary Ca:P ratio | | | | | | |
|---|---|---|---|---|---|---|---|
| Measure | 1:2[be] | 1:4[bf] | 1:1[ce] | 1:2[cf] | 2:1[de] | 1:1[df] | SEM |
| Urine pH | 6.36[k] | 6.22[k] | 6.50[k] | 6.26[k] | 6.88[l] | 6.36[k] | .076 |
| Urine Ca (mg/dl) | 3.0[k] | 2.4[k] | 3.1[k] | 2.7[k] | 6.1[l] | 3.6[k] | .28 |
| Urine P (mg/dl) | 185 | 275 | 151 | 302 | 112 | 221 | 22.2[h] |
| Plasma Ca (mg/dl) | 9.6 | 10.1 | 9.8 | 10.1 | 10.1 | 10.6 | .15[h] |
| Plasma inorg. P (mg/dl) | 6.0[k] | 7.6[l] | 5.6[k] | 6.2[ik] | 6.0[k] | 5.3[jk] | .24 |
| Plasma ALP (U/L)[g] | 18.2 | 22.8 | 18.8 | 21.5 | 18.2 | 19.6 | 1.61[h] |

[a]Eight observations per mean.
[b].3% Ca, [c].6% Ca, [d]1.2% Ca (dietary DM).
[e].6% P, [f]1.2% P (dietary DM).
[g]One unit of activity defined as that amount of enzyme which will produce 1 micromole of p-nitrophenol per min under conditions of Sigma assay procedure #245.
[h]Ca x P interaction not significant (NS).
[ij]Means within a row with different letter superscripts are significantly different (P <.05).
[kl]Means within a row with different letter superscripts are significantly different (P <.01).

the urine and plasma values measured, it was difficult to assess the independent effects of dietary Ca and P concentrations, Ca:P ratios or $H_3PO_4$ additions. Thus, a second experiment was conducted to more closely examine the effects of $H_3PO_4$ additions to the diets of ferrets.

Experiment 1 provided some evidence as to the efficacy of $H_3PO_4$ as a urinary acidifier. In Experiment 2, various concentrations of dietary $H_3PO_4$ were examined in attempts to more closely identify an optimal level for maximum urinary acidification.

In Experiment 2, the Ca concentration of the test diets was held constant to, hopefully, reduce the variation which may have been due to the different Ca concentrations in the diets of Experiment 1.

The trial period was extended to 42-d in Experiment 2 and samples of urine and blood were obtained more often. Male ferrets only were used to reduce variation which may have been due to sex. A new variable, age, was also introduced in Experiment 2.

## Experiment 2

### Materials and Methods

**A. Animals and procedures**

A total of 24 male ferrets, 12 mature (18 months of age) and 12 young (5 months of age), were used. Ferrets were blocked by age and randomly assigned to one of the four test diets. All animals were housed under similar conditions as described in Experiment 1. The ferrets were allowed a 2-d adjustment period to the new diets. Food and water were provided ad libitum, fresh daily, for a 42-d trial period.

On a biweekly basis, the ferrets were placed in individual metabolism cages for a 3-d sampling period to obtain urine output as well as feed intake. Each day during the sampling period, urine pH was measured, within a few hours of being voided, at approximately 12:00 noon using a glass electrode pH meter. A previous investigation indicated no change in ferret or mink urine pH which remained at room temperature for 5 hr. Daily urine volumes were recorded, and the 3-d samples were filtered through cheese-cloth to remove sediment, pooled for a 25-ml aliquot, and a few drops of toluene were added before being frozen for later analyses.

At this same 2-week interval, ferrets were anesthetized and a 10-ml blood sample was drawn as described in Experiment 1. The heparinized blood was centrifuged for 15 minutes at 2100 x g. Plasma was then separated and stored in plastic tubes at -20C until further analyses were performed.

B. Diet composition

Four diets were prepared from the basal diet shown in Table 1. All diets contained approximately 31% dry matter, 32% protein and 11% fat on a dry matter basis. The calcium concentration was held constant at .6% of the diets on a dry matter basis. Phosphoric acid (85% $H_3PO_4$) was added at the expense of alpha-cellulose at either 0, .75, 1.86 or 2.98% to bring the phosphorus concentration to .4, .6, .9 or 1.2% of dietary dry matter, respectively. The resulting calcium to phosphorus ratios for the control (0% $H_3PO_4$) and the other three treatment diets were 1.5:1, 1:1, 1:1.5 and 1:2, respectively.

C. Laboratory analyses

Urine

Frozen urine samples were thawed and the specific gravities were calculated by determination of mass per volume (density of urine/density of $H_2O$ =sp gr). A 10-ml sample was digested with 5 ml nitric acid and 1 ml perchloric acid (wet oxidation) in a 50 ml Phillips beaker. After the acid digestion was completed, distilled deionized $H_2O$ was added to bring the volume to 50 ml. To a 1-ml sample of this dilution was added 2 ml of 10,000 ppm $SrCl_2$ for calcium analysis by atomic absorption spectrophotometry (IL Model 951, Instrumentation Laboratory Inc., Wilmington, MA). A .1 ml volume of the diluted, digested urine was further diluted with .9 ml $H_2O$ and analyzed for phosphorus by the method of

Table 1. Composition of Ferret Diets (Experiment 2)

| BASAL DIET | |
|---|---|
| Ingredient | % of dietary DM |
| Ground beef | 63.00 |
| Cereal by-product[a] | 12.00 |
| Dextrose[b] | 18.00 |
| Calcium carbonate | 1.52 |
| MSU vitamin-trace mineral premix[c] | .50 |
| Vitamin E premix[d] | .05 |
| Selenium premix[e] | .05 |
| | 95.12 |

**TEST DIETS**

Treatment 1 (control): Basal + 4.88% alpha-cellulose[f]
Treatment 2: Basal + 4.13% alpha-cellulose + .75% $H_3PO_4$[g]
Treatment 3: Basal + 3.02% alpha-cellulose + 1.86% $H_3PO_4$
Treatment 4: Basal + 1.90% alpha-cellulose + 2.98% $H_3PO_4$

[a]Kel-Mix, Kellogg Co., Battle Creek, MI.
[b]Cerelose, Corn Products Co., Argo, IL.
[c]Vitamin concentrations (per kg): retinyl acetate, 660,000 USP units; cholecalciferol, 132,000 USP units; riboflavin, 660 mg; d-calcium pantothenate, 2,640 mg; nicotinic acid, 3,520 mg; cyanocobalamin, 3.96 mg; choline chloride, 25,344 mg; menadione sodium bisulfite complex, 440 mg. Mineral concentrations in % (and source): zinc, 1.496 (zinc oxide); iron, 1.188 (ferrous sulfate); manganese, .748 (manganous oxide); copper, .198 (copper oxide); iodine, .06 (potassium iodate).
[d]500,000 USP units supplied per kg.
[e]200 mg Se supplied per kg.
[f]Solka-Floc, Brown Co., Boston, MA.
[g]85% phosphoric acid.

Fiske and Subbarow (1925). Urine creatinine was determined using Sigma procedure #555 (Sigma Diagnostics, St. Louis, MO) on a Beckman Gilford spectrophotometer (Model 2400, Beckman Instruments, Inc., Fullerton, CA).

Plasma

A 1-ml sample was mixed with 4 ml trichloroacetic acid (12.5%) and centrifuged for 15 minutes at 900 x g. The deproteinized supernatant was transferred to plastic tubes for storage. This supernatant was diluted 1:2 with 20,000 ppm of $SrCl_2$ and analyzed for calcium by atomic absorption spectrophotometry. Phosphorus determination was performed by the method of Fiske and Subbarow (1925). The plasma creatinine concentrations and alkaline phosphatase (ALP) activities were measured by Sigma procedures #555 and #245, respectively.

D. Statistical analyses

All data were subjected to analysis of variance by the SAS general linear model procedures appropriate for a split-plot design (SAS Institute Inc., Cary, NC).

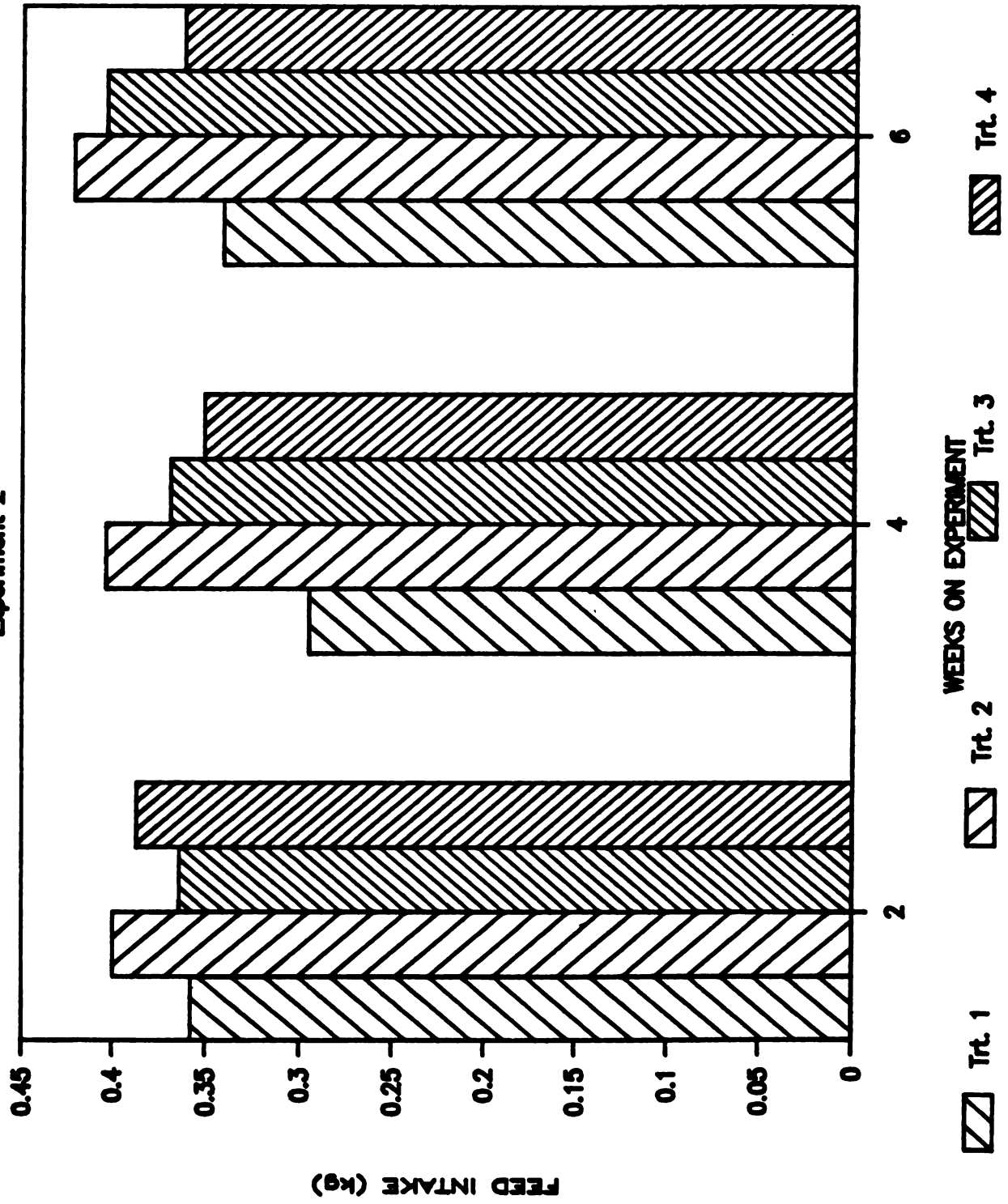
## Results and Discussion

A. Feed intake and weight gain

There was no statistically significant deleterious effect from any of the dietary $H_3PO_4$ additions on feed intake (Figure 1). In fact, the control group (Treatment 1)

**Figure 1.** **The effects, over time, of phosphoric acid added at 0, .75, 1.86 or 2.98% of the dietary dry matter on average daily feed intake (as fed basis) of ferrets (Experiment 2).**

# FIGURE 1. AVG DAILY FEED INTAKE



had an average daily feed intake consistently lower than the other groups. All animals gained weight until wk 4, but mean body weight for all groups then decreased slightly by wk 6 (Figure 2). Treatment group 3 had a mean body weight which was consistently greater than the others in each period. Ferrets on Treatment 3 (1.86% $H_3PO_4$) had the highest gain:feed ratio of 12.73 g/kg, followed by Treatment 2 , Treatment 1 and, finally, Treatment 4 (Table 2). Age had no effect on either body weight or feed intake.

B. Urine

Urinary pH values for the 6-wk trial are presented in Table 3. Animals consuming the Treatment 1 diet, with no $H_3PO_4$ addition, had fluctuating urine pH values. There was an increase in pH from 6.12 initially to 7.37 after 2 wk. At 4 wk, urine pH had decreased to 7.08 but rose again to 7.31 after 6 wk on the experiment. Treatment group 2 (.75% $H_3PO_4$) exhibited a slight increase from an initial pH of 6.22 to 6.41 at 2 wk, and increased again to 6.51 at 4 wk. At the 6 wk sampling period, mean urinary pH for Treatment 2 decreased to 6.43. There was only a slight decrease of urine pH in Treatment group 3 (1.86% $H_3PO_4$) at 4 wk (6.06), and again at 6 wk (6.03). Treatment group 4 (2.98% $H_3PO_4$) had a mean urine pH initially of 6.27 which decreased to 6.01 at 2 wk. By wk 4, there was a rapid decline to a mean urine pH of 5.86 that persisted through wk 6 (Figure 3).

By the second wk of the experiment, all diets with $H_3PO_4$ additions had mean urinary pH values that were lower (P

**Figure 2.** **The effects, over time, of phosphoric acid added at 0, .75, 1.86 or 2.98% of the dietary dry matter on body weights of ferrets (Experiment 2).**

# FIGURE 2. BODY WEIGHT

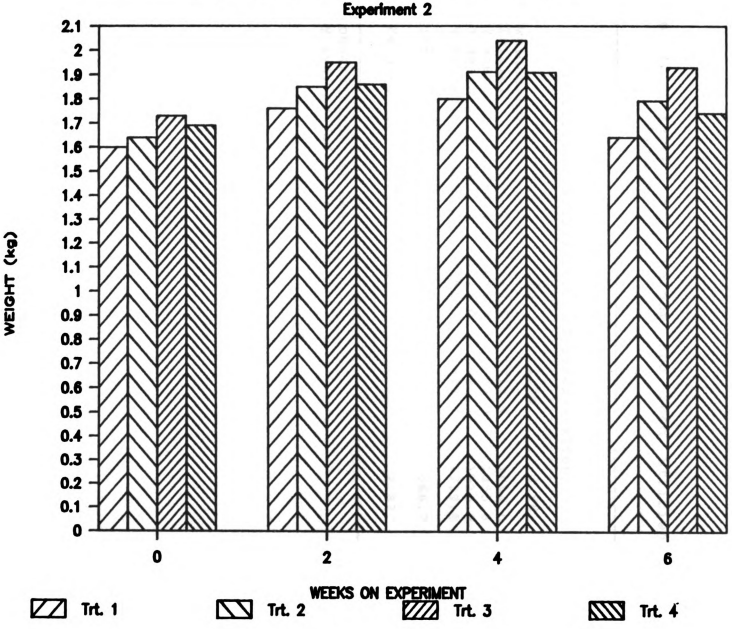


Table 2. Ferret Body Weight and Feed Efficiency[a] (Experiment 2)

| | Dietary $H_3PO_4$ (% of dry matter) | | | |
|---|---|---|---|---|
| | .0 | .75 | 1.86 | 2.98 |
| Body weight (g) | | | | |
| Initial | 1599.3 | 1640.5 | 1729.8 | 1694.0 |
| 2 Weeks | 1764.3 | 1851.3 | 1955.3 | 1855.8 |
| 3 Weeks | 1802.2 | 1912.0 | 2040.7 | 1910.2 |
| 4 Weeks | 1643.0 | 1791.7 | 1932.7 | 1739.8 |
| Feed intake[b] (g/d) | 331.5 | 408.7 | 379.3 | 366.7 |
| Gain:Feed (g/kg) | 3.14 | 8.81 | 12.73 | 2.97 |

[a]Six observations per mean.
[b]Daily avg, as fed basis.

Table 3. Phosphoric Acid Additions to Diets for Ferrets and Effects, Over Time, on Urine pH[ab] (Experiment 2)

| | Dietary $H_3PO_4$ (% of dry matter) | | | | | |
|---|---|---|---|---|---|---|
| | .0 | .75 | 1.86 | 2.98 | SEM | P< |
| Week on trial | | | | | | |
| 0 | 6.12 | 6.22 | 6.10[c] | 6.27[d] | .036 | .05 |
| 2 | 7.37[e] | 6.41[cf] | 6.11[df] | 6.01[df] | .062 | .001 |
| 4 | 7.08[e] | 6.51[f] | 6.06[g] | 5.88[g] | .068 | .001 |
| 6 | 7.31[e] | 6.43[f] | 6.03[c] | 5.86[dg] | .032 | .001 |

[a]Six observations per mean.

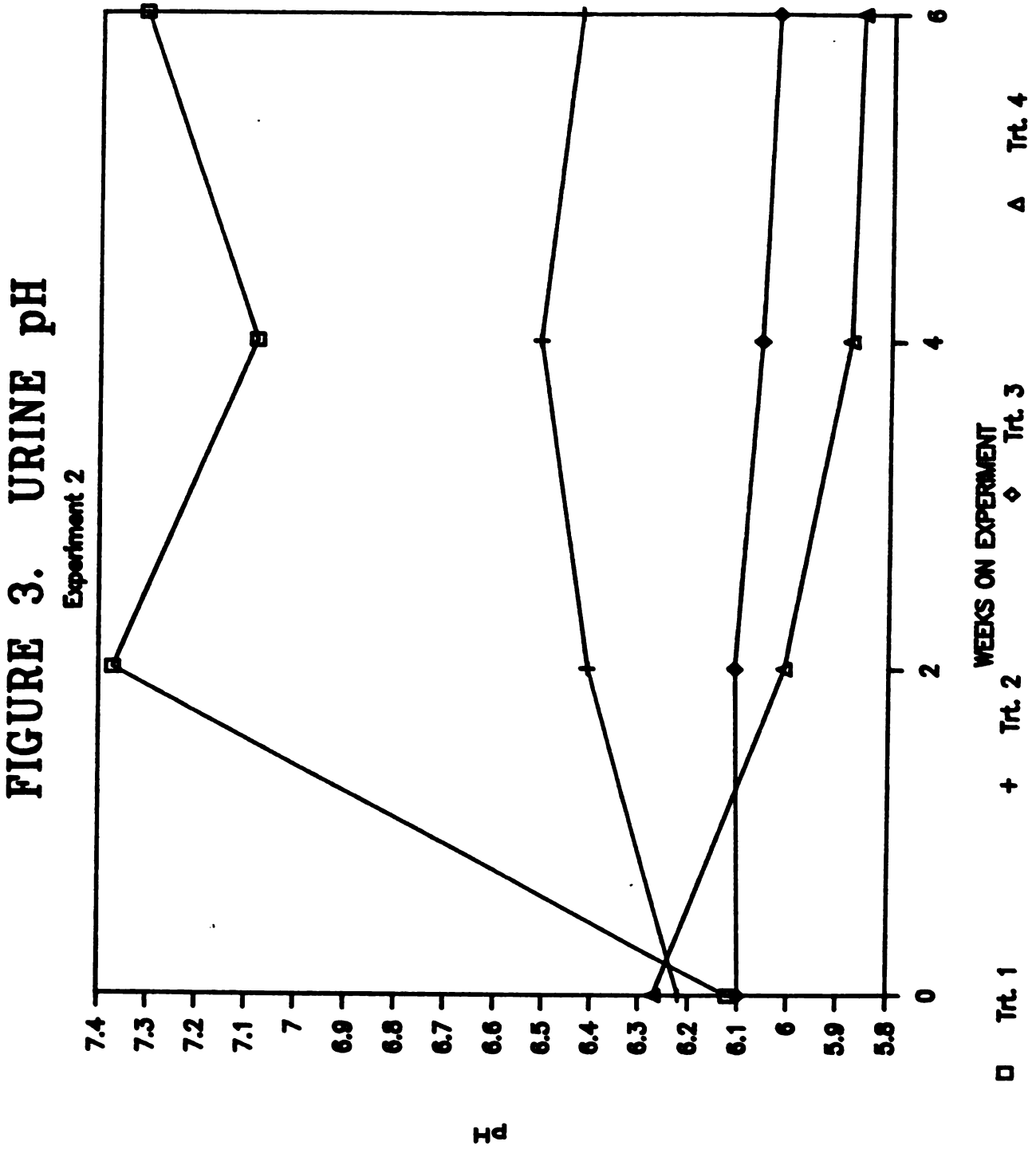
[b]Significant (P <.001) treatment (SEM .640), time (SEM .024) and treatment x time effects.

[cd]Means within a row with different letter superscripts are significantly different (P <.01).

[efg]Means within a row with different letter superscripts are significantly different (P <.001).

Figure 3. The effects, over time, of phosphoric acid added at 0, .75, 1.86 or 2.98% of the dietary dry matter on urine pH of ferrets. SEM and significant differences are presented in Table 3 (Experiment 2).

# FIGURE 3. URINE pH



<.001) than those in the control group, and these differences persisted throughout the rest of the trial. Treatment 4 consistently produced the lowest pH values, and these were significantly lower (P <.001) than those on the other treatments after 4 wk.

Urine pH of mink fed 1 g $NH_4Cl$/d has been reported to be in the range of 5.5 to 5.9 (Leoschke and Elvehjem, 1955). Senior et al. (1986) determined that a dose of 800 mg/d of $NH_4Cl$ was sufficient to decrease urine pH of the cat from 6.42 to 5.90. These values are similar to the urinary pH values observed in ferrets when they were fed diets containing 2.98% added $H_3PO_4$. Reductions of urinary pH to 6.0 or less would be sufficient acidification to prevent precipitation of struvite crystals even if the urine were supersaturated (Senior et al., 1986). Feeding a diet that consistently maintains a urine pH of less than 6.4 has been said to not only aid in the prevention of urolithiasis, but also to be beneficial in dissolving struvite uroliths, and to reduce the incidence of recurrence (Lewis et al., 1987). If this is true, dietary additions of $H_3PO_4$, at a level between 1.86 and 2.98% of the dry matter, would aid in the prevention of struvite urolithiasis in the ferret by means of urinary acidification.

There was a significant period effect but no significant treatment effect on urine Ca concentration (Table 4). Urinary Ca concentration of the control group remained fairly constant throughout the duration of the experiment. Urine Ca concentrations are presented in Figure 4. All diets with

Table 4. Phosphoric Acid Additions to Diets for Ferrets and Effects, Over Time, on Urine Concentrations of Calcium and Phosphorus, and on Volume and Specific Gravity[a] (Experiment 2)

| Urine measure | Week on trial | Dietary $H_3PO_4$ (% of dry matter) .0 | .75 | 1.86 | 2.98 | SEM | P< |
|---|---|---|---|---|---|---|---|
| Ca (mg/dl)[b] | | | | | | | |
| | 0 | 3.0 | 3.6 | 3.4 | 3.2 | .42 | NS |
| | 2 | 2.2 | 2.6 | 2.6 | 2.4 | .26 | NS |
| | 4 | $2.1^{h}$ | $2.4^{f}$ | $3.0^{gi}$ | 2.7 | .19 | .05 |
| | 6 | 2.2 | 2.0 | 2.6 | 2.0 | .19 | NS |
| P (mg/dl)[c] | | | | | | | |
| | 0 | 222 | 171 | 219 | 204 | 13.3 | NS |
| | 2 | $77^{j}$ | $205^{k}$ | $365^{l}$ | $587^{m}$ | 12.6 | .001 |
| | 4 | $79^{j}$ | $111^{j}$ | $248^{k}$ | $354^{l}$ | 14.9 | .001 |
| | 6 | $63^{j}$ | $295^{k}$ | $485^{l}$ | $920^{m}$ | 15.8 | .001 |
| Volume (ml)[d] | | | | | | | |
| | 0 | 77.4 | 96.0 | 78.0 | 71.3 | 7.84 | NS |
| | 2 | $142.4^{h}$ | $185.5^{fi}$ | $158.2^{g}$ | 162.9 | 7.73 | .01 |
| | 4 | $122.8^{h}$ | $189.2^{i}$ | 157.5 | $158.8^{i}$ | 11.90 | .01 |
| | 6 | $128.9^{fj}$ | $179.6^{k}$ | $159.5^{g}$ | $158.9^{g}$ | 8.64 | .01 |
| Specific gravity[e] | | | | | | | |
| | 0 | $1.03^{f}$ | $1.19^{g}$ | $1.04^{f}$ | $1.07^{f}$ | .040 | .05 |
| | 2 | $1.04^{f}$ | $1.03^{g}$ | $1.03^{g}$ | $1.04^{f}$ | .003 | .05 |
| | 4 | $1.04^{f}$ | $1.03^{g}$ | 1.04 | $1.04^{f}$ | .004 | .05 |
| | 6 | 1.00 | .99 | 1.03 | 1.04 | .076 | NS |

[a] Six observations per mean.

[b] Treatment effect nonsignificant (NS). Significant time effect (P <.001). Treatment x time NS.

[c] Significant (P <.001) treatment (SEM 23.9), time (SEM 22.0) and treatment x time effects.

[d] Significant (P <.01) treatment (SEM 7.050) and time (SEM 3.398) effects. Treatment x time significant (P <.10).

[e] Significant (P <.10) treatment (SEM .022) and (P <.01) time (SEM .021) effects. Treatment x time significant (P <.10).

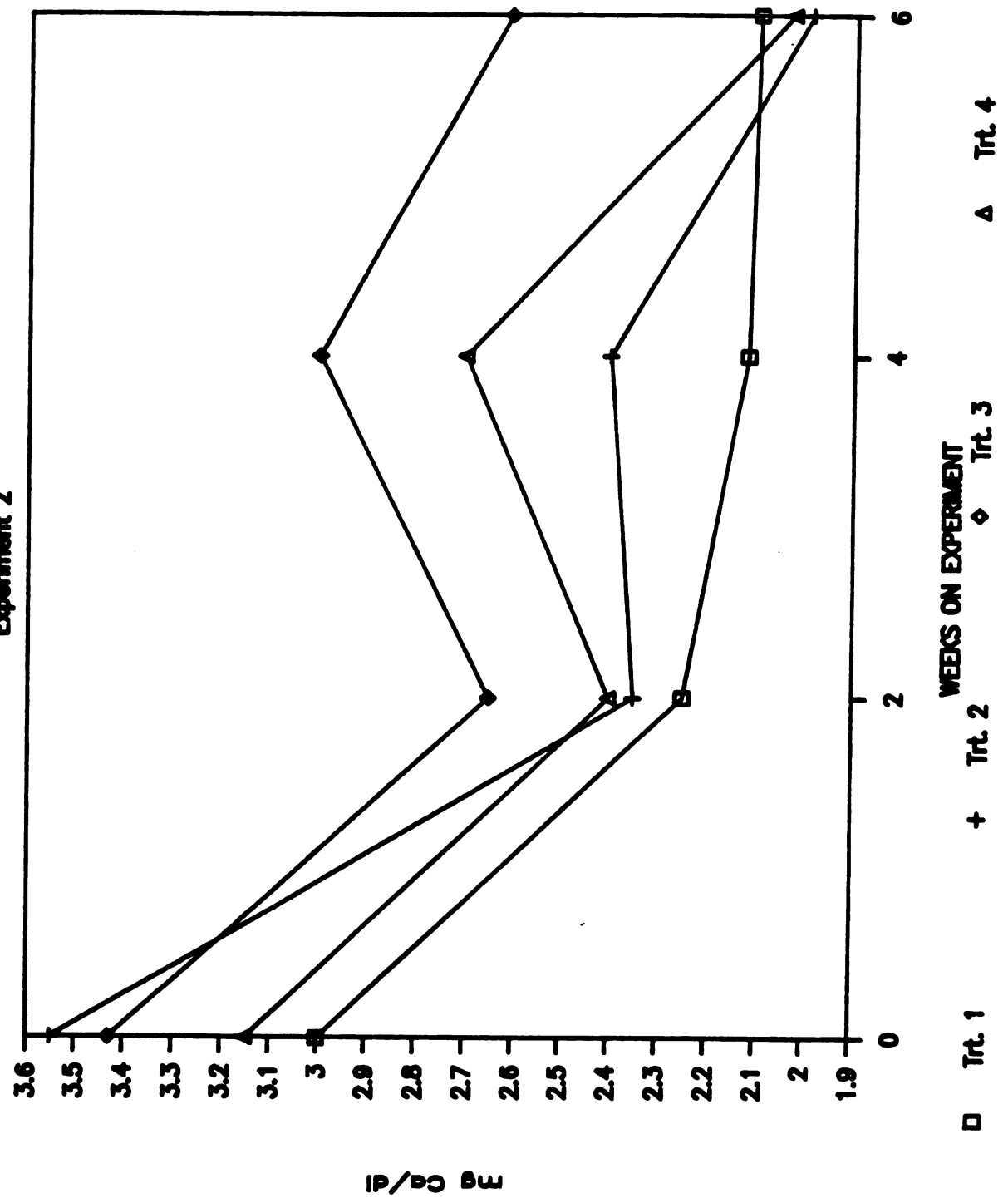
[fg] Means within a row with different letter superscripts are significantly different (P <.05).

[hi] Means within a row with different letter superscripts are significantly different (P <.01).

[jklm] Means within a row with different letter superscripts are significantly different (P <.001).

Figure 4. The effects, over time, of phosphoric acid added at 0, .75, 1.86 or 2.98% of the dietary dry matter on urine calcium concentrations of ferrets. SEM and significant differences are presented in Table 4 (Experiment 2).

# FIGURE 4. URINE Ca CONCENTRATION



$H_3PO_4$ additions resulted in similar trends in urinary Ca excretion, with an ultimate decrease in urine Ca concentration from the initial sample to the 6-wk sample. It appears, therefore, that dietary $H_3PO_4$ was successful in reducing urinary Ca losses by 6 wk.

Aulerich (1981) reported Ca concentrations in urine excreted by mink on stock diets to be approximately 5.2 mg/dl. Values for urine Ca excreted by ferrets during this experiment were lower, ranging from 2.0 to 3.6 mg/dl. Since there were no increases in urinary Ca concentration or excretion, the addition of $H_3PO_4$ to the diets of ferrets, even at 2.98% of the dry matter, did not appear, in the short term, to induce the metabolic acidosis which has been seen in some animals fed other acid substances (Goto, 1918; Farquharson et al., 1930; Petito and Evans, 1984).

Urine P concentrations rose in accordance with increasing levels of dietary $H_3PO_4$ (Table 4), which would be expected since the kidney is the main control mechanism for P homeostasis (Irving, 1964). Normal average values for urine P excreted by the cat and mink are $294\pm45$ and $256\pm41$ mg/dl, respectively (Finco et al., 1985; Aulerich, 1981). The control group had below normal urinary P concentrations after 2 wk of the experiment. All animals fed diets with $H_3PO_4$ additions exhibited increased mean urinary P concentrations over time as compared to their initial values. After 2 wk on the experiment, ferrets in each treatment group had greater ($P < .001$) urinary P concentrations than in the urine of

ferrets receiving the next lower dietary $H_3PO_4$ concentration. At 4 wk, urine P concentrations decreased but rose again at 6 wk in all groups fed $H_3PO_4$. Once again, at increasing concentrations of dietary $H_3PO_4$, the urinary P concentrations were greater ($P < .001$) than in urine of ferrets receiving the next lower dietary $H_3PO_4$ concentration. It is interesting that as urinary Ca increased during the trial, urinary P decreased and vice-versa, for ferrets consuming $H_3PO_4$ at any concentration (Figures 4 and 5). The reason for this is not completely clear but may be due to renal adaptation and homeostatic mechanisms.

All treatment groups exhibited approximately a 2-fold increase in mean urine volume after 2 wk of consuming the experimental diets (Figure 6). At wk 4, the control group (0% $H_3PO_4$) had a lower ($P < .05$) urine volume than the groups fed $H_3PO_4$. This trend remained significant at wk 6. Treatment groups 3 and 4 excreted similar volumes of urine at the 2-, 4- and 6-wk sampling times. Animals on Treatment 2 (.75% $H_3PO_4$) had consistently higher urine volumes, though only marginally significant ($P < .10$), as compared to Treatment groups 3 and 4 at wk 4 (Table 4).

Increasing urine volumes will decrease the concentration of calculi-forming constituents in the urine and may also increase the frequency of urination (Lewis et al., 1987). Dietary additions of $H_3PO_4$ may, therefore, reduce the potential for urolith formation by increasing urine volume of ferrets.

Figure 5. The effects, over time, of phosphoric acid added at 0, .75, 1.86 or 2.98% of the dietary dry matter on urine phosphorus concentrations of ferrets. SEM and significant differences are presented in Table 4 (Experiment 2).

# FIGURE 5. URINE P CONCENTRATION

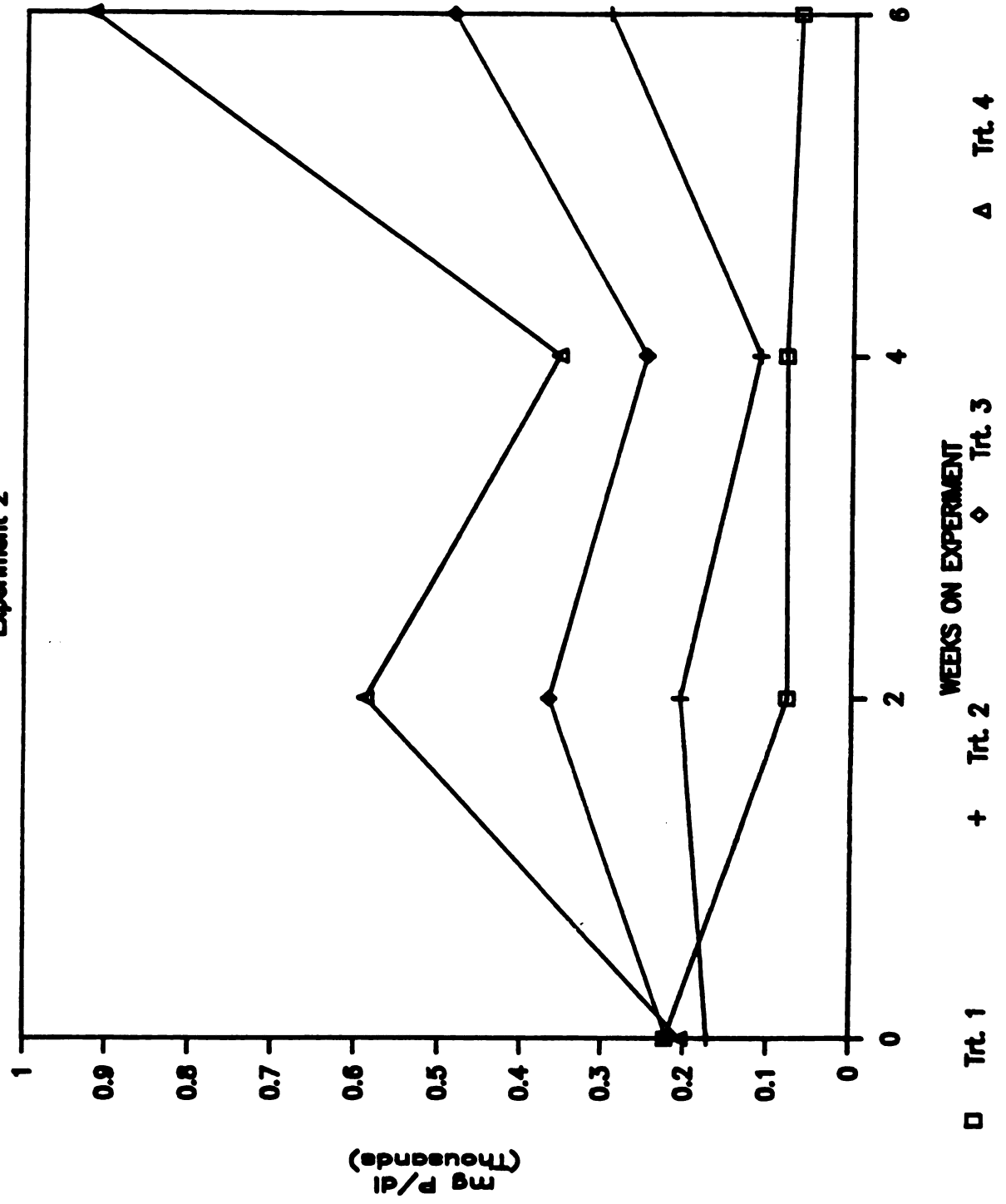
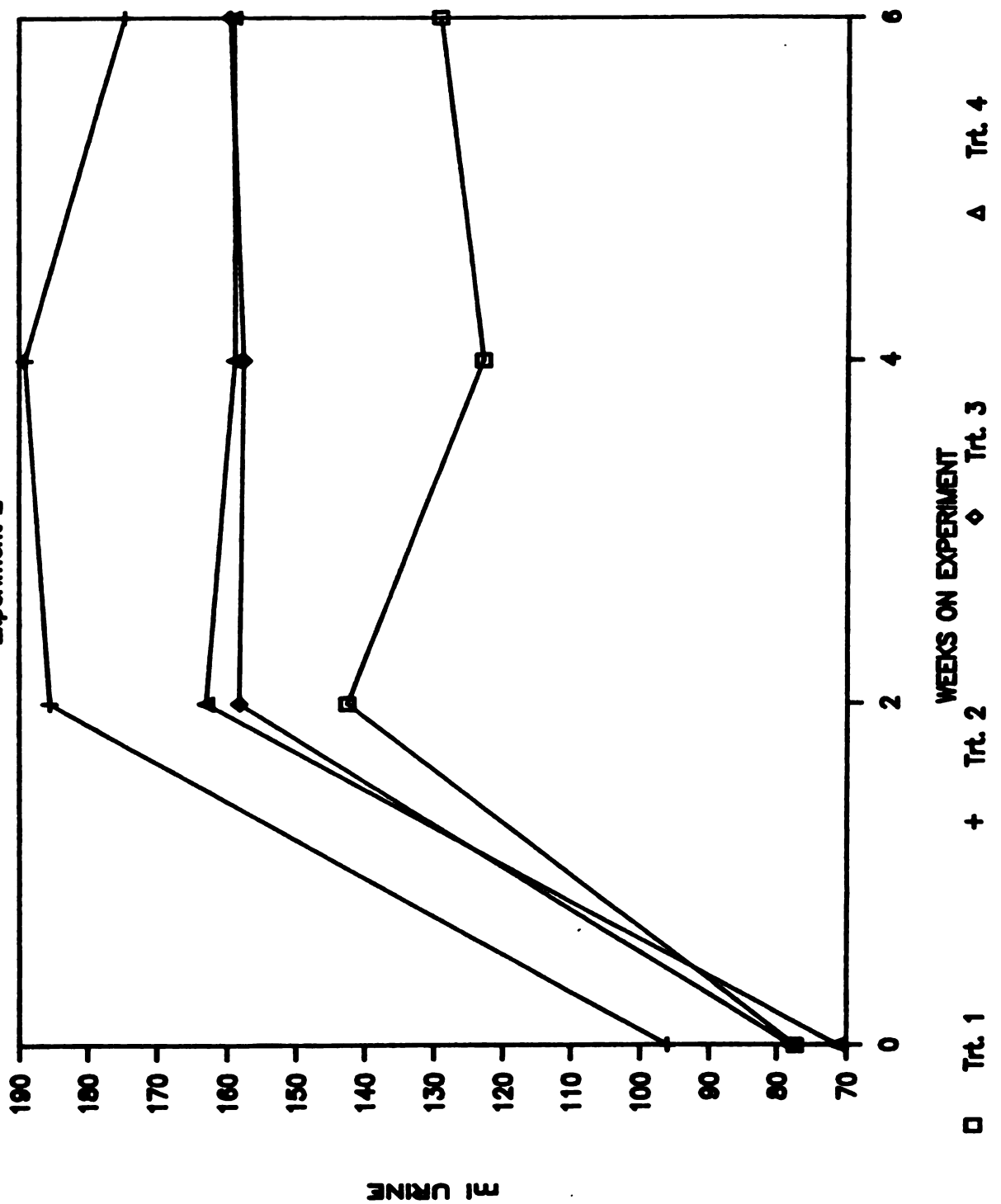


Figure 6. The effects, over time, of phosphoric acid added at 0, .75, 1.86 or 2.98% of the dietary dry matter on daily urinary excretion volumes of ferrets. SEM and significant differences are presented in Table 4 (Experiment 2).

# FIGURE 6. URINE VOLUME



Water intake measures were attempted during the 3-d sampling periods when the ferrets were in metabolism cages. Unfortunately, all too often, the ferrets would pull the cork and nipple from the $H_2O$ bottle, or simply push the bottle from its holder to the floor. Thus, it was difficult to measure water intakes with confidence. Data from water consumption measures were too inconsistent to correlate with either feed intake or urine volume.

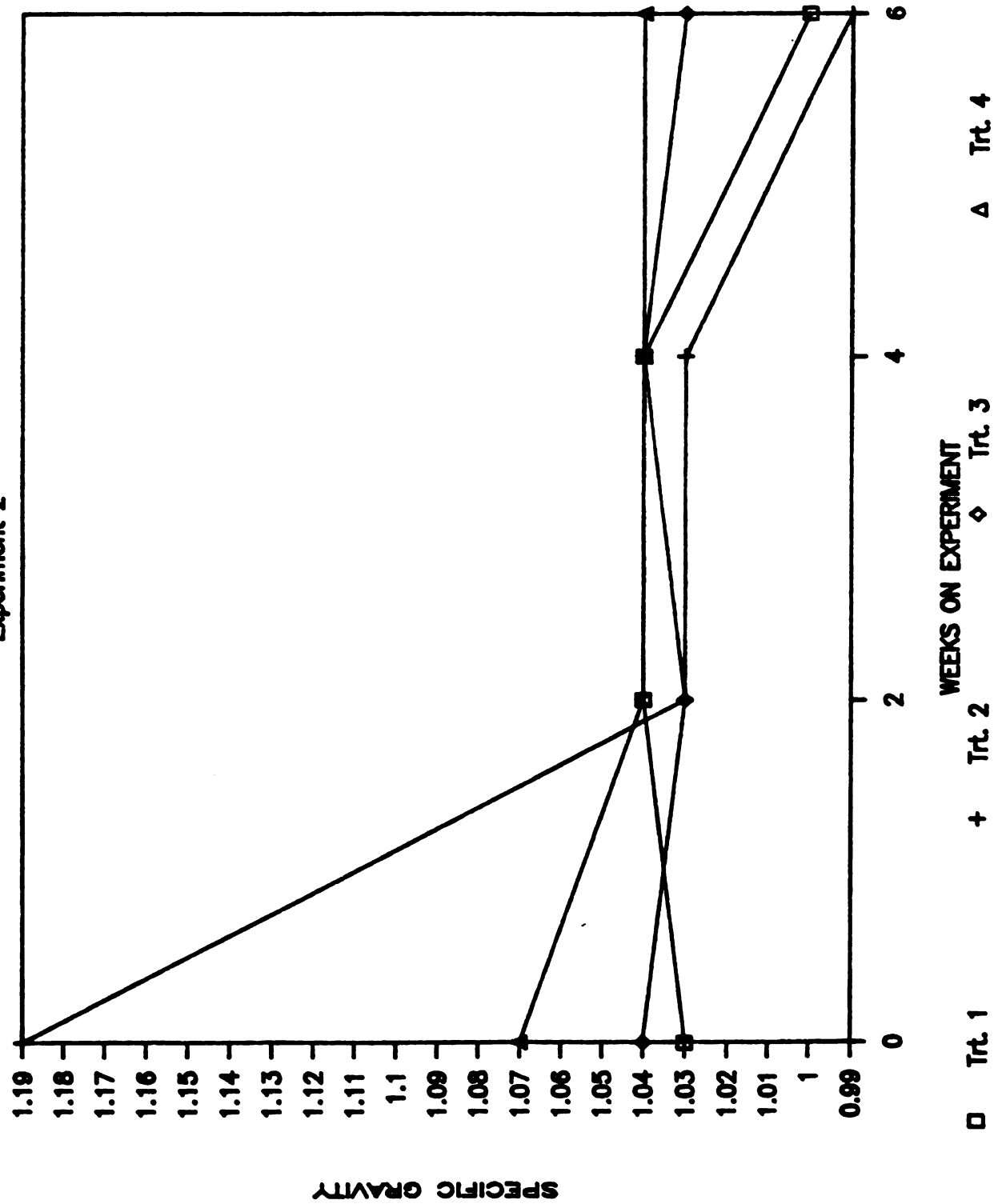
All treatment groups fed $H_3PO_4$ had decreased urine specific gravity values from the initial to the 2 wk sampling time (Figure 7). The range for ferret urine specific gravity has been reported to be 1.028 to 1.050 (Hoover and Baldwin, 1987). The urine specific gravities of Treatments 1 and 2 decreased below the above range after 6 wk of the experiment. The specific gravity of urine of ferrets on Treatments 3 and 4 remained fairly constant and within the above limits for the duration of the trial.

Normal specific gravity values for feline urine have been reported in some textbooks (Kaneko, 1980; Bloom, 1960) to be in the range of 1.02 to 1.04. However, Palmore and co-workers (1978) observed feline urine specific gravity may be as high as 1.078. This hypersthenuria can be explained, in part, by the cat's ability to concentrate its urine, which is further enhanced when diets high in protein are consumed. Evolution of the carnivora has given these species long renal papillae which allow for a great capacity for water conservation and urine concentration (Palmore et al., 1978).

Urinary measurements are summarized in Tables 3 and 4.

**Figure 7.** **The effects, over time, of phosphoric acid added at 0, .75, 1.86 or 2.98% of the dietary dry matter on urine specific gravities of ferrets. SEM and significant differences are presented in Table 4 (Experiment 2).**

# FIGURE 7. URINE SPECIFIC GRAVITY



C. Plasma

The trend for all groups, except Treatment group 3, was to exhibit a rise in plasma Ca concentration after 2 wk (Figure 8). Ferrets consuming 1.86% $H_3PO_4$ in the diet (Treatment 3) actually had a decline in plasma Ca concentration after 2 wk. By wk 4, there were no significant differences among the groups (Table 5). At wk 6 of the experiment, Treatment group 2 (.75% $H_3PO_4$) had a greater ($P < .01$) mean plasma Ca concentration than the other groups. All values were within the normal range, which is reported to be 8.3 to 11.8 mg/dl for male ferrets (Thornton et al., 1979). Therfore, theses changes may not be physiologically important.

Throughout the experiment, all ferrets maintained plasma inorganic P concentrations within the normal range of 4.0 to 7.9 mg/dl (Thornton et al., 1979) (Table 5). All treatment groups exhibited an increase in plasma inorganic P concentration after 2 wk of the trial, and declined thereafter (Figure 9). At the 6- wk sampling, Treatment groups 3 and 4 once again exhibited a slight increase. At the conclusion of the trial, Treatment group 4 had a mean plasma inorganic P concentration which was greater ($P < .05$) than that of Treatments 1 and 2.

The increase in urinary P concentrations when ferrets were fed $H_3PO_4$, while maintaining normal inorganic P concentrations in the plasma, is evidence that the ferret's homeostatic mechanisms are capable of adapting to an acid

Figure 8. The effects, over time, of phosphoric acid added at 0, .75, 1.86 or 2.98% of the dietary dry matter on plasma calcium concentrations of ferrets. SEM and significant differences are presented in Table 5 (Experiment 2).

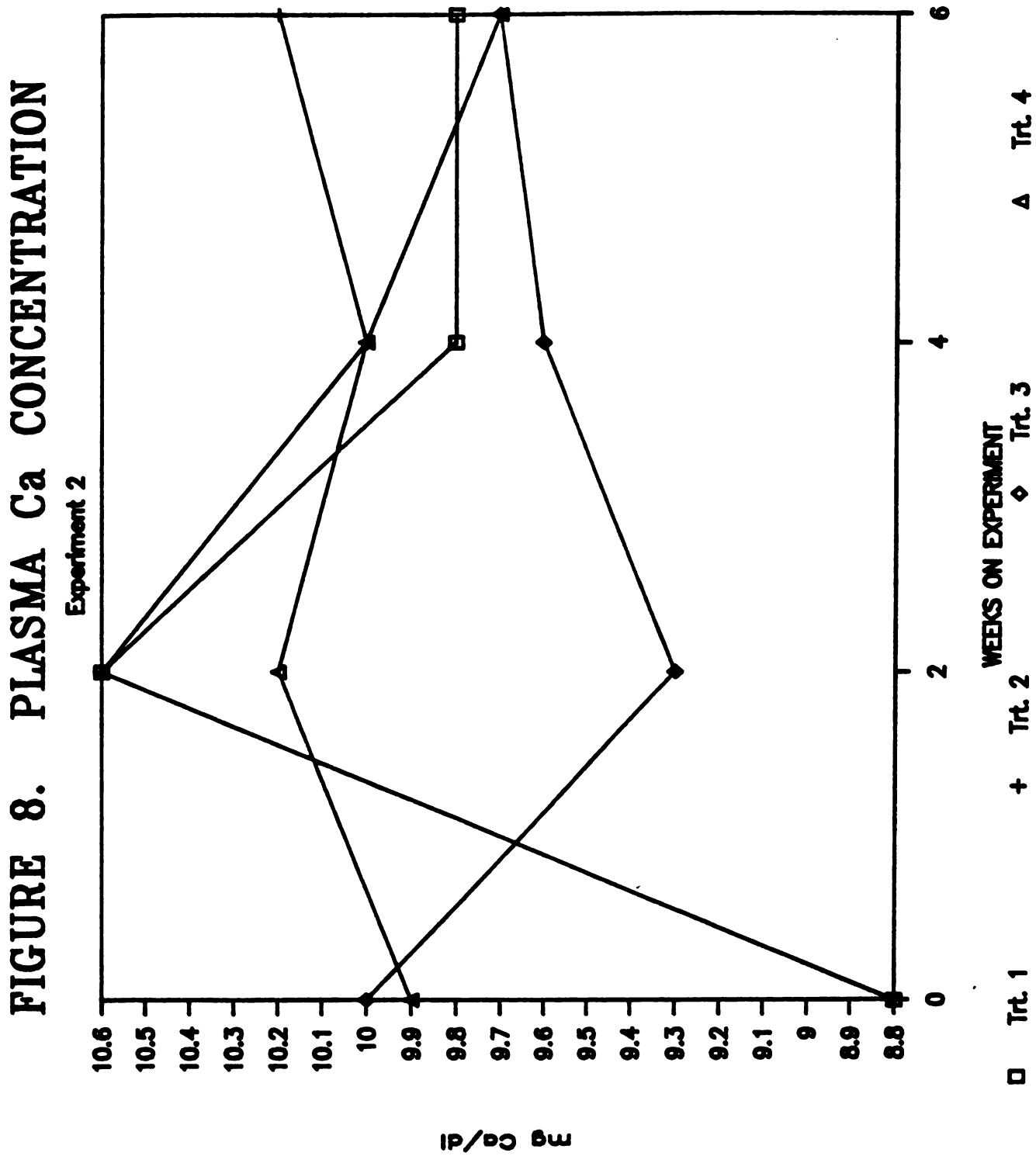


FIGURE 8. PLASMA Ca CONCENTRATION
Experiment 2
mg Ca/dl
10.6
10.5
10.4
10.3
10.2
10.1
10
9.9
9.8
9.7
9.6
9.5
9.4
9.3
9.2
9.1
9
8.9
8.8
0
2
4
6
WEEKS ON EXPERIMENT
□ Trt. 1
+ Trt. 2
◇ Trt. 3
△ Trt. 4

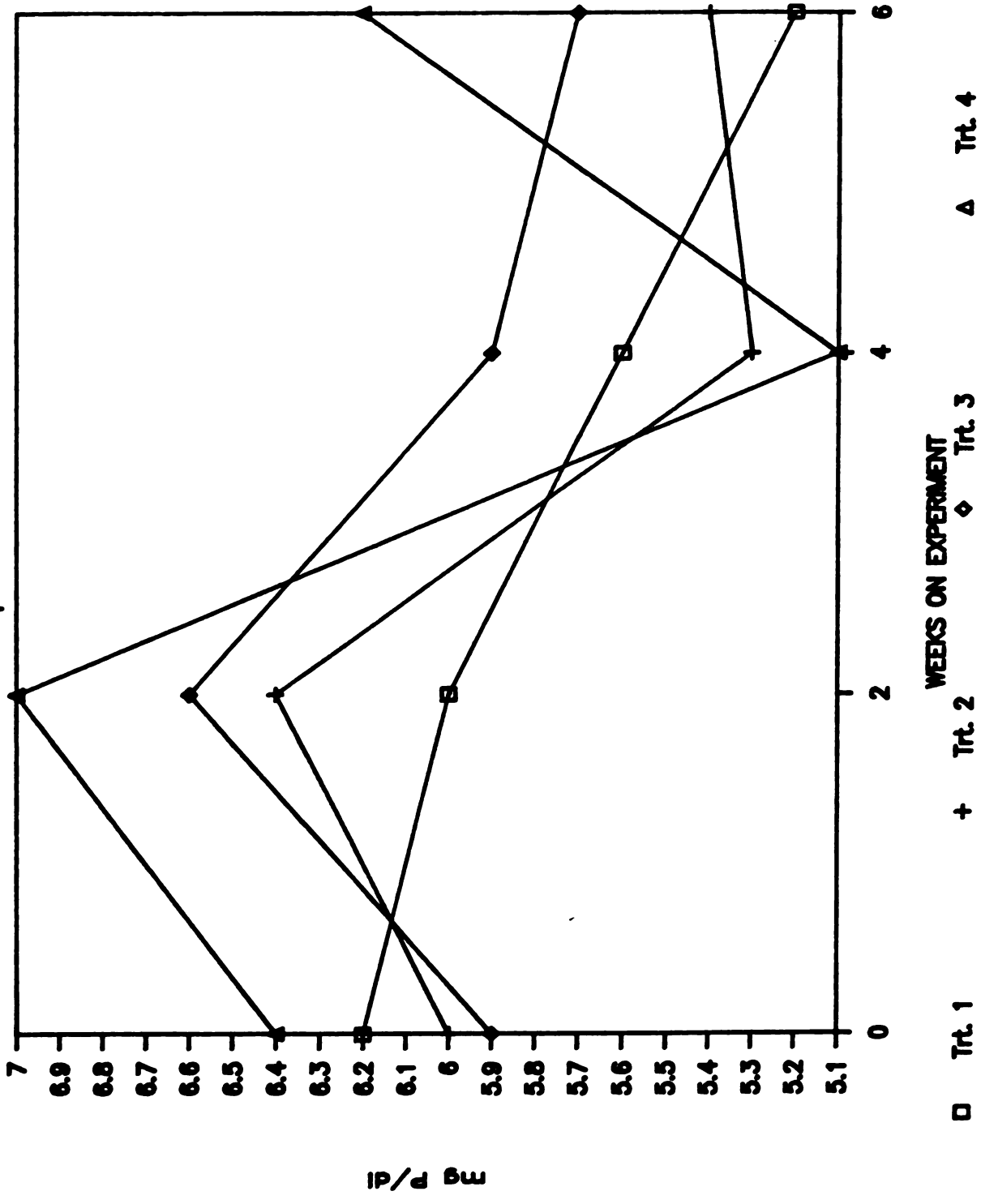
Table 5. Phosphoric Acid Additions to Diets for Ferrets and Effects, Over Time, on Plasma Concentrations of Calcium, Inorganic Phosphorus and Creatinine and on Alkaline Phosphatase Activity[a] (Experiment 2)

| Plasma measure | Week on trial | Dietary $H_3PO_4$ (% of dry matter) .0 | .75 | 1.86 | 2.98 | SEM | P< |
|---|---|---|---|---|---|---|---|
| Ca (mg/dl)[b] | | | | | | | |
| | 0 | 8.8[fh] | 8.8[fh] | 10.0[i] | 9.9[g] | .28 | .01 |
| | 2 | 10.6[f] | 10.7[f] | 9.3[g] | 10.2 | .34 | .05 |
| | 4 | 9.8 | 10.0 | 9.6 | 10.0 | .11 | NS |
| | 6 | 9.8[h] | 10.2[i] | 9.7[h] | 9.7[h] | .07 | .001 |
| Inorg. P (mg/dl)[c] | | | | | | | |
| | 0 | 6.2 | 6.0 | 5.9 | 6.4 | .29 | NS |
| | 2 | 6.0 | 6.4 | 6.6 | 7.0 | .38 | NS |
| | 4 | 5.6 | 5.3 | 5.9 | 5.1 | .23 | NS |
| | 6 | 5.2[h] | 5.4[f] | 5.7 | 6.2[gi] | .23 | .05 |
| Creatinine (mg/dl)[d] | | | | | | | |
| | 0 | 1.47[h] | 1.54[h] | 1.04[i] | 1.02[i] | .090 | .001 |
| | 2 | 1.06[h] | 1.08[h] | 1.26[i] | 1.23[i] | .032 | .001 |
| | 4 | .98 | 1.01 | 1.07 | 1.11 | .059 | NS |
| | 6 | 1.24[fh] | 1.28 | 1.32[g] | 1.34[i] | .020 | .05 |
| ALP (U/L)[e] | | | | | | | |
| | 0 | 12.2 | 13.2 | 13.5 | 13.2 | 1.42 | NS |
| | 2 | 11.5[h] | 15.6[i] | 16.3[i] | 18.2[i] | .99 | .01 |
| | 4 | 20.5[f] | 23.3 | 22.8 | 25.8[g] | 1.28 | .10 |
| | 6 | 17.3[f] | 21.0 | 18.7 | 22.9[g] | 1.56 | .10 |

[a]Six observations per mean.
[b]Treatment effect nonsignificant (NS) (SEM .30). Significant (P <.001) time effects (SEM .38) and treatment x time.
[c]Significant (P <.05) treatment (SEM .36) and time (SEM .48) effects. Treatment x time NS.
[d]Treatment effect NS (SEM .031). Significant (P <.01) time (SEM .028) and treatment x time effects.
[e]One unit of activity defined as that amount of enzyme which will produce 1 micromole of p-nitrophenol per min under the conditions of Sigma assay procedure #245. Significant (P <.05) treatment (SEM .96) effect. Significant (P <.001) time (SEM .53) effect. Treatment x time NS.
[fg]Means within a row with different letter superscripts are significantly different (P <.05).
[hi]Means within a row with different letter superscripts are significantly different (P <.01).

Figure 9. The effects, over time, of phosphoric acid added at 0, .75, 1.86 or 2.98% of the dietary dry matter on plasma phosphorus concentrations of ferrets. SEM and significant differences are presented in Table 5 (Experiment 2).

# FIGURE 9. PLASMA P CONCENTRATION



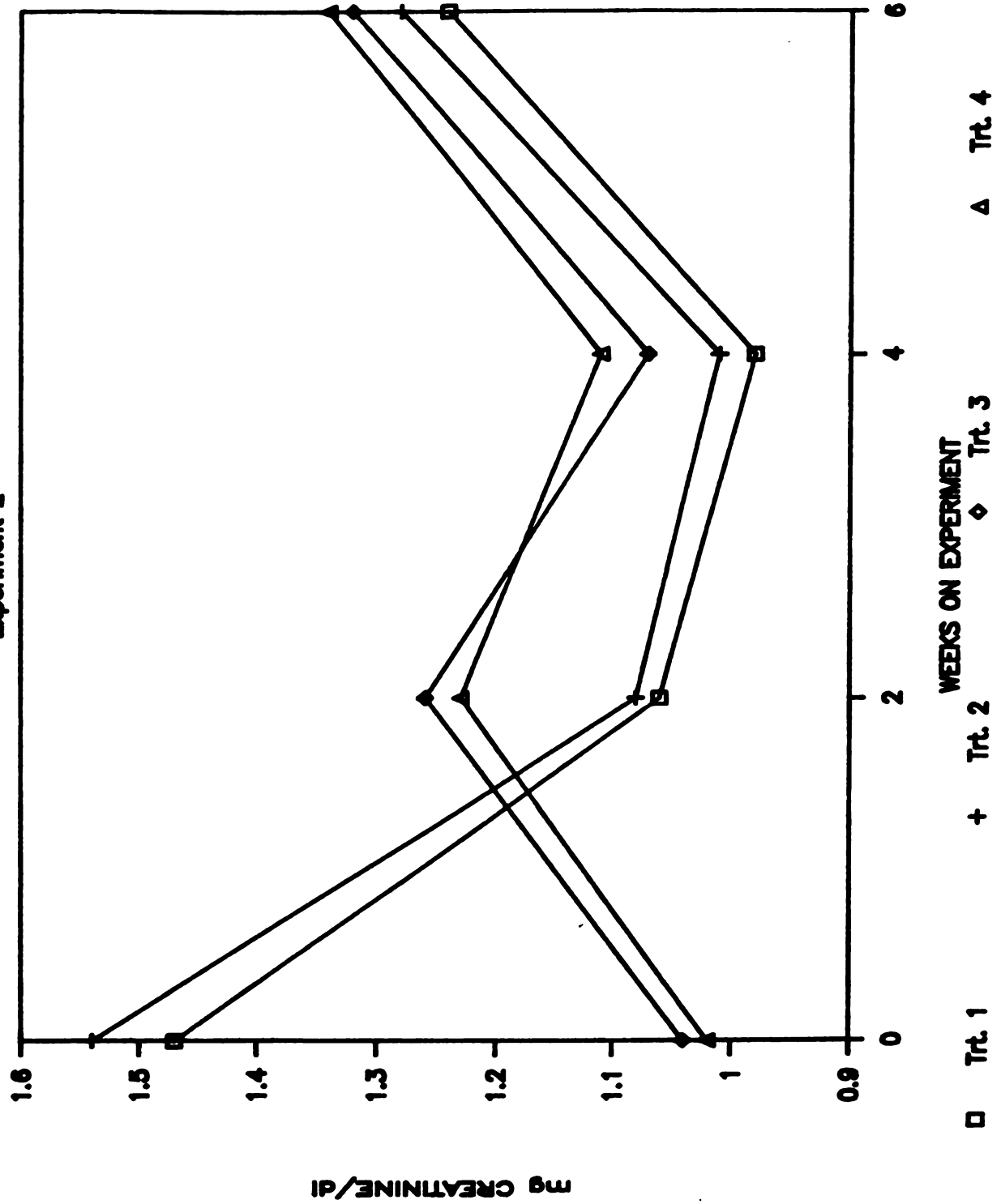
load. Unlike in the rat (Sie et al., 1974), high dietary P did not increase plasma inorganic P nor decrease plasma Ca in the ferret, which is suspected of leading to bone resorption via parathyroid hormone secretion. Since there was no indication of hyperphosphatemia, the dietary concentrations of P were probably not beyond the ability of the ferret to accomodate, at least in the short term. Renal adaptation appeared to be the control mechanism in ferrets consuming $H_3PO_4$ in their diets.

The normal range for plasma creatinine in ferrets has been reported to be .5 to 1.1 mg/dl (Hoover and Baldwin, 1987). Plasma creatinine concentrations were elevated above this range in all animals throughout the experiment (Table 5). The plasma creatinine concentrations of Treatment groups 1 and 2 followed a similar pattern, whereas groups 3 and 4 followed another (Figure 10). At 6 wk, animals receiving no dietary $H_3PO_4$ (Treatment group 1) had lower ($P < .05$) plasma creatinine concentrations than animals receiving 1.86% or 2.98% $H_3PO_4$ (Treatments 3 and 4, respectively).

In most domestic species, serum concentrations of creatinine are crude estimates of glomerular filtration rate. Serum creatinine concentrations may exceed the normal range when renal dysfunction occurs. However, it has been emphasized (Finco, 1980) that measurement of serum creatinine concentration alone is not a precise method of assessing existing renal function. Because the creatinine concentrations in the ferrets' plasma were only slightly

Figure 10. The effects, over time, of phosphoric acid added at 0, .75, 1.86 or 2.98% of the dietary dry matter on plasma creatinine concentrations of ferrets. SEM and significant differences are presented in Table 5 (Experiment 2).

# FIGURE 10. PLASMA CREATININE



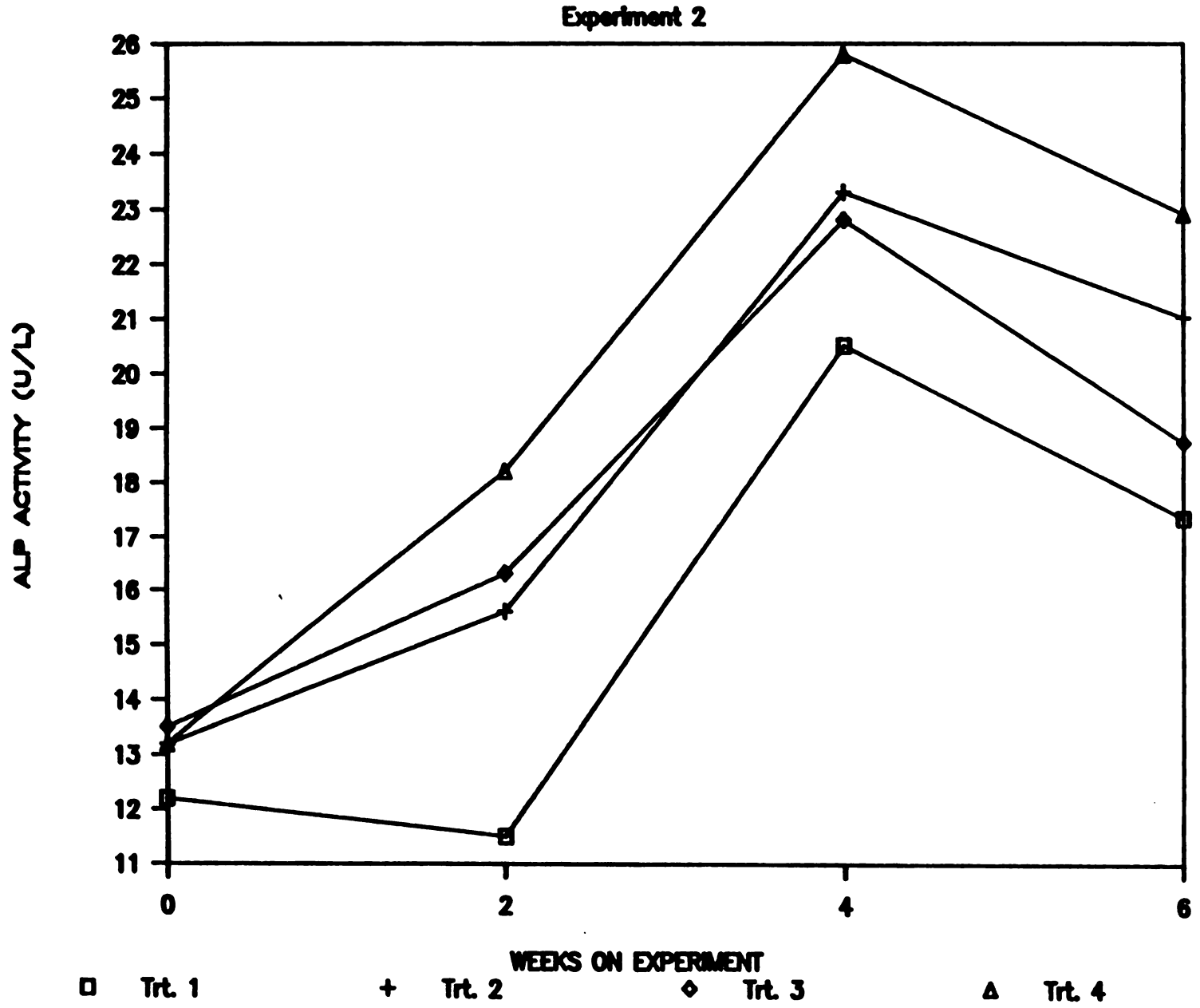
elevated above normal, it is assumed that there were no cases of renal dysfunction resulting from the consumption of $H_3PO_4$.

Plasma ALP activities (Figure 11) of all animals were below mean values previously reported, though still within the normal range of 11 to 84 U/L (Thornton et al., 1979). Age had a significant ($P < .01$) effect in that, during each period, young animals had a higher mean ALP activity than the mature animals. This was expected since data presented by Hoover and Baldwin (1987) showed a decline in mean serum ALP from 129 U/L in ferrets 12 wk of age, to 16 U/L in ferrets almost 1 yr old. Growing bone contains high ALP activity. Therefore, due to the high osteoblastic activity in bone of young animals, serum ALP activity would be expected to be greater than in mature animals (Kramer, 1980). The relatively low and normal plasma ALP activities of the ferrets on all the treatments were an indication that the diets were supplying adequate available P. It is unlikely that the low plasma ALP values encountered were due to hypothyroidism, malnutrition or severe chronic nephritis (Cohn and Kaplan, 1966), since a wide range of activity is acceptable in the ferret.

Phosphoric acid appears to be an effective urinary acidifier for the ferret. Furthermore, there were no detectable adverse effects of high dietary levels of $H_3PO_4$ fed to ferrets on plasma Ca, P, and creatinine concentrations, nor on ALP activity. Even when the $H_3PO_4$ additions resulted in dietary P concentrations which were above the requirement, the ferret was able to adapt and

Figure 11. The effects, over time, of phosphoric acid added at 0, .75, 1.86 or 2.98% of the dietary dry matter on plasma alkaline phosphatase activities of ferrets. SEM and significant differences are presented in Table 5 (Experiment 2).

# FIGURE 11. PLASMA ALKALINE PHOSPHATASE



maintain Ca and P homeostasis in the plasma. The kidney was able to cope with the high P concentrations, and excess Ca loss via the urine did not occur.

## EXPERIMENT 3

Calcium and P are two very important minerals found in the body. Calcium is a vital component of bones and teeth, which contain approximately 99% of the body's Ca (NRC, 1980). The Ca found in extracellular fluid is critical for muscle contractility, normal neuromuscular excitability, blood coagulation, nerve impulse transmission, myocardial function and capillary and cell membrane permeability (Simesen, 1980). Eighty-five percent of the body's P is also found in the bones and teeth. The remaining P is distributed between tissues and membrane components where it is involved in energy utilization, phospholipid formation, protein formation and maintenance of acid-base balance (Avioli, 1988).

There is a paucity of data concerning the carnivore's qualitative and minimal quantitative requirements for minerals (MacDonald et al., 1984). Calcium and P requirements have been established for the cat and mink. The cat requires 1.0% Ca and .8% P, providing a dietary Ca to P ratio of 1.25:1 (NRC, 1986). Growing mink require .4 to 1.0% Ca and .4 to .8% P, providing vitamin D levels are adequate , resulting in a Ca to P ratio between .75:1 and 1.7:1 (NRC, 1982). The nutritional requirements of ferrets have not been determined but are assumed to be similar to those of the cat and mink since other aspects of their nutrient metabolism are similar (Ryland et al., 1983).

Skeletal muscle has relatively low Ca and high P concentrations, approximately .03% and .6% of the dry matter,

respectively (McCullough and Ullrey, 1983; Bennett, 1976). Therefore, optimal Ca to P ratios would not be met by feeding an all-meat diet to a carnivore. That carnivorous animals eat flesh, is not to imply that they do not consume other body parts of their prey. In order to obtain all necessary dietary minerals, a carnivore must consume some bone, cartilage or other mineralized tissue.

Imbalances of dietary Ca and P are known to cause numerous severe disorders in animals. Nutritional secondary hyperparathyroidism results from a diet containing a relative excess of P and insufficient Ca, ultimately leading to widespread bone resorption (Palmore, 1968). Rickets, a disease associated with young growing animals, occurs when there is an inadequate dietary supply of Ca and/or P and/or vitamin D. The result is a failure of Ca salts to be deposited in the bone matrix. In the mature animal, a similar lack of these nutrients results in osteomalacia, manifested by reabsorption of bone mineral already laid down in the matrix (Simesen, 1980). Calcium and P may also be involved in the development of osteoporosis, although the precise etiology of this disease is still unknown. With this disease, there is a net resorption of bone tissue (Simesen, 1980). There is a decrease in total bone mass while the ratio of minerals to organic matrix appears normal.

Despite extensive research, much controversy exists concerning the roles of dietary Ca, P, protein and acidity in the development of osteoporosis. Early suggestions that

chronic Ca deficiency was a major cause of osteoporosis were refuted when Ca administration failed to reverse bone loss (Draper and Scythes, 1981). High dietary P intakes have been shown to have both adverse and beneficial effects with respect to osteoporosis. Studies with aged rats and mice have shown that excess P intakes decrease Ca and P concentrations in the bones (Draper, 1972; Draper et al., 1972; Anderson and Draper, 1972). On the other hand, a high P intake has also been reported to decrease urinary Ca excretion, possibly due to a decrease in bone resorption or to an increase in bone formation and mineralization (Goldsmith et al., 1976; Spencer et al., 1978b).

High dietary protein has been proposed as a possible contributory factor in the etiology of osteoporosis due to increased Ca excretion in the urine and a negative Ca balance when purified diets, high in casein, were fed to rats (Johnson et al., 1970; Walker and Linkswiler, 1972). However, Spencer and co-workers (1978b and 1983) did not observe increases in urinary Ca excretion when human subjects were fed high protein diets composed primarily of meat. Diets which are highly acidic have also been reported to increase bone resorption, leading to skeletal osteopenia (Milligan and Evans, 1978).

Experiments 1 and 2 examined the effects of dietary $H_3PO_4$ and various Ca and P levels on selected urine and plasma values of adult ferrets. Dietary factors such as a low Ca or a high P intake, which may cause an increase in bone resorption by stimulating parathyroid production, have a

greater effect on the skeleton of younger animals than of adults (Draper and Scythes, 1981). Basset et al. (1951) concluded that the ratio of Ca to P in the diet is the most important consideration in determining the requirements for Ca and P of growing mink.

Thus, young growing ferrets were utilized in this trial to compare the relative effects of various dietary Ca to P ratios on the growth, and physical and/or chemical properties of plasma and bone. Narrow Ca:P ratios were selected in order to examine the ferret's sensitivity to these two elements. Extreme deviations from a 1:1 Ca to P ratio would undoubtedly elicit abnormal development. It was hypothesized, however, that the ferret would be able to tolerate slight departures from the 1:1 ratio which is usually regarded as optimal for other species. Considering the natural dietary habits of carnivores, encounters with inverse Ca:P ratios do not seem unrealistic. The ferret was employed as a model carnivore to investigate possible metabolic effects and/or adaptations to a dietary Ca:P ratio less than one.

This experiment was approved by the AUCAUC of MSU.

## Experiment 3

### Materials and Methods

**A. Animals and procedures**

Sixty ferrets, 30 males and 30 females, approximately 8 weeks of age were involved in this experiment. After a 3-d adjustment period, the ferrets were randomly assigned to one of six dietary treatments so that each group contained five ferrets of both sexes. All animals were housed under conditions previously described.

The ferrets were provided food and $H_2O$ ad libitum for a 42-d trial period. Feed intake was estimated weekly by the methods described in Experiment 1. Body weight and length (from tip of nose to rump) were measured weekly.

At the conclusion of the 6-wk experiment, all ferrets were anesthetized and a 10-ml blood sample was drawn with a heparinized syringe in the manner described for the previous experiments. The heparinized blood was centrifuged for 15 minutes at 2100 x g. Plasma was then separated and put into plastic tubes for storage at -20C until further analyses could be performed.

Six animals from each treatment group, three males and three females, were randomly chosen and killed with carbon dioxide gas inhalation. Left and right femurs were removed and cleaned of muscle, carefully avoiding removal of hyaline cartilage. The femurs were weighed fresh, and measurements of total and shaft (distance between epiphyseal plates)

lengths were measured with vernier calipers. Midpoint shaft diameter was measured in two directions, anterior to posterior (A-P) and lateral to medial (L-M). Femurs were sealed in plastic bags and frozen (-20C) for later analyses.

B. Diet composition

A 2 x 3 factorial design was used, with three dietary Ca concentrations and two Ca:P ratios. Diets were formulated to contain .6, .7, or .8% Ca (dry matter basis), resulting in a Ca:P ratio of 1.3:1 or 1:1.3 (Table 1). Phosphoric acid was used as the supplemental source of P. Upon analysis, the diets contained approximately 33% dry matter, 32% crude protein and 19% fat. The dietary Ca concentrations were within the range of requirements for the mink and cat. The dietary P concentrations were within or above those recommended for mink and cats (NRC, 1982; NRC, 1986).

C. Laboratory analyses

Plasma

Plasma Ca and P concentrations were determined by methods as previously described. Plasma creatinine concentrations and alkaline phosphatase (ALP) activities were measured on a Beckman Gilford spectrophotometer (Model 2400, Beckman Instruments Inc., Fullerton, CA) using Sigma procedures #555 and #245, respectively (Sigma Diagnostics, St. Louis, MO).

Table 1. Composition of Ferret Diets (Experiment 3)

| | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Ingredient | % of dietary DM | | | | | |
| Ground beef | 63.0 | 63.0 | 63.0 | 63.0 | 63.0 | 63.0 |
| Cereal by-product[a] | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| Dextrose[b] | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 | 22.0 |
| Calcium carbonate | 1.53 | 1.53 | 1.79 | 1.79 | 2.05 | 2.05 |
| Phosphoric acid (85%) | 0 | 1.18 | .30 | 1.67 | .60 | 2.15 |
| Alpha-cellulose[c] | 4.87 | 3.69 | 4.31 | 2.94 | 3.75 | 2.20 |
| MSU VTM premix[d] | .50 | .50 | .50 | .50 | .50 | .50 |
| Vitamin E premix[e] | .05 | .05 | .05 | .05 | .05 | .05 |
| Selenium premix[f] | .05 | .05 | .05 | .05 | .05 | .05 |
| %Ca | .6 | .6 | .7 | .7 | .8 | .8 |
| %P | .46 | .78 | .54 | .91 | .62 | 1.04 |
| Ca:P | 1.3:1 | 1:1.3 | 1.3:1 | 1:1.3 | 1.3:1 | 1:1.3 |

[a]Kel-Mix, Kellogg's Inc., Battle Creek, MI.
[b]Cerelose, Corn Products Co., Argo, IL.
[c]Solka-Floc, Brown Co., Boston, MA.
[d]MSU vitamin-trace mineral premix. Vitamin concentrations (per kg): retinyl acetate, 660,000 USP units; cholecalciferol, 132,000 USP units; riboflavin, 660 mg; d-calcium pantothenate, 2,640 mg; nicotinic acid, 3,520 mg; cyanocobalamin, 3.96 mg; choline chloride, 25,344 mg; menadione sodium bisulfite complex, 440 mg. Mineral concentrations in % (and source): zinc, 1.496 (zinc oxide);iron, 1.188 (ferrous sulfate); manganese, .748 (manganous oxide); copper, .198 (copper oxide); iodine, .06 (potassium iodate).
[e]500,000 USP units supplied per kg.
[f]200 mg Se supplied per kg.

**Femurs**

A portable device consisting of a stainless steel base and adjustable fulcra (adjustable in both length and height) was built to accomodate the small ferret femurs. The maximum force required to break the thawed, fresh bones was determined using an Instron Universal Testing Instrument (Model 4202, Instron Corp., Canton, MA). The fulcra were adjusted to suit each bone when the breaking force was measured so that the center of the femur shaft received the force applied by the Instron. Bending moment was then calculated using the formula M =Wl/4, where W is maximal force and l is the distance between fulcra (Miller et al., 1962).

The broken femurs were tied into bundles with ashless filter paper and string , and the water and fat extracted in a Soxhlet apparatus with ethanol for 20 hr followed by anhydrous diethyl ether for 20 hr. They were then dried overnight in a vacuum oven at 60C. The dry, fat-free femurs were ashed in a muffle furnace at 600C overnight. Between .1 and .15 g of the bone ash was digested with 5 ml of 6 N HCl and then diluted with 100 ml of distilled deionized $H_2O$ for mineral analyses. Femur P concentration was determined by the colormetric method of Fiske and Subbarow (1925). Sodium (Na) and potassium (K) concentrations were measured in the diluted ash samples by atomic absorption spectrophotometry (IL Model 951, Instrumentation Laboratories Inc., Wilmington, MA). A .2-ml sample of the diluted ash was mixed with 3.8 ml of 10,000 ppm $SrCl_2$ for Ca and magnesium (Mg) analyses by

atomic absorption spectrophotometry.

D. Statistical analyses

Data were analyzed by SAS general linear model procedures using least square means (SAS Institute Inc., Cary, NC).

## Results and Discussion

A. Feed intake and growth

Average daily feed intake is presented in Table 2. There was no statistically significant effect of dietary Ca:P ratio on feed intake. Since the animals were growing, their feed intake was expected to increase and this was confirmed by a significant ($P < .001$) period effect. There was also a significant ($P < .05$) effect of dietary Ca concentration on feed intake. However, there was not one treatment group that consistently had the highest or lowest intake throughout the trial. Calcium supplied at .7% of the dietary dry matter (Treatments 3 and 4) produced the lowest gain:feed overall.

Figures 1 and 2 illustrate mean ferret body weight and body length at the 2-, 4- and 6-wk measurement intervals. There were no significant effects of either dietary Ca or Ca:P ratios on the growth of the ferrets (Table 3). Both male and female ferrets in all treatment groups were at or above body weights and lengths of ferrets at similar ages reported previously (Shump and Shump, 1978).

The ferrets readily consumed all diets and grew

Table 2. Average Daily Feed Intake if Ferrets Fed Various Levels of Dietary Calcium and Resultant Calcium to Phosphorus Ratios[a] (Experiment 3)

| | Dietary Ca (% of dry matter) | | | | | |
|---|---|---|---|---|---|---|
| | .6 | | .7 | | .8 | |
| | Ca:P ratio | | | | | |
| | 1.3:1 | 1:1.3 | 1.3:1 | 1:1.3 | 1.3:1 | 1:1.3 |
| Feed intake (g dry matter/d) | | | | | | |
| Week 1 | 68.1 | 69.2 | 70.0 | 77.2 | 67.0 | 70.4 |
| Week 2 | 63.5 | 66.1 | 64.9 | 78.4 | 61.6 | 64.7 |
| Week 3 | 64.1 | 65.3 | 67.5 | 77.3 | 64.4 | 64.7 |
| Week 4 | 58.5 | 59.1 | 71.3 | 74.0 | 66.5 | 65.0 |
| Week 5 | 77.5 | 71.9 | 71.9 | 76.2 | 69.8 | 67.7 |
| Week 6 | 103.9 | 106.7 | 101.8 | 107.5 | 103.9 | 101.8 |
| Average daily intake | 72.6 | 73.0 | 74.5 | 81.7 | 72.2 | 72.1 |
| Gain:Feed (g/kg) | 135.1 | 140.2 | 121.4 | 121.2 | 128.9 | 139.0 |

[a]Ten observations per mean.

Figure 1. The effects of calcium at .6, .7 or .8% of the dietary dry matter and a calcium to phosphorus ratio of 1.3:1 or 1:1.3, over time, on the body weights of growing ferrets. Treatment effect NS (SEM 53.81). (Experiment 3).

# FIGURE 1. BODY WEIGHT

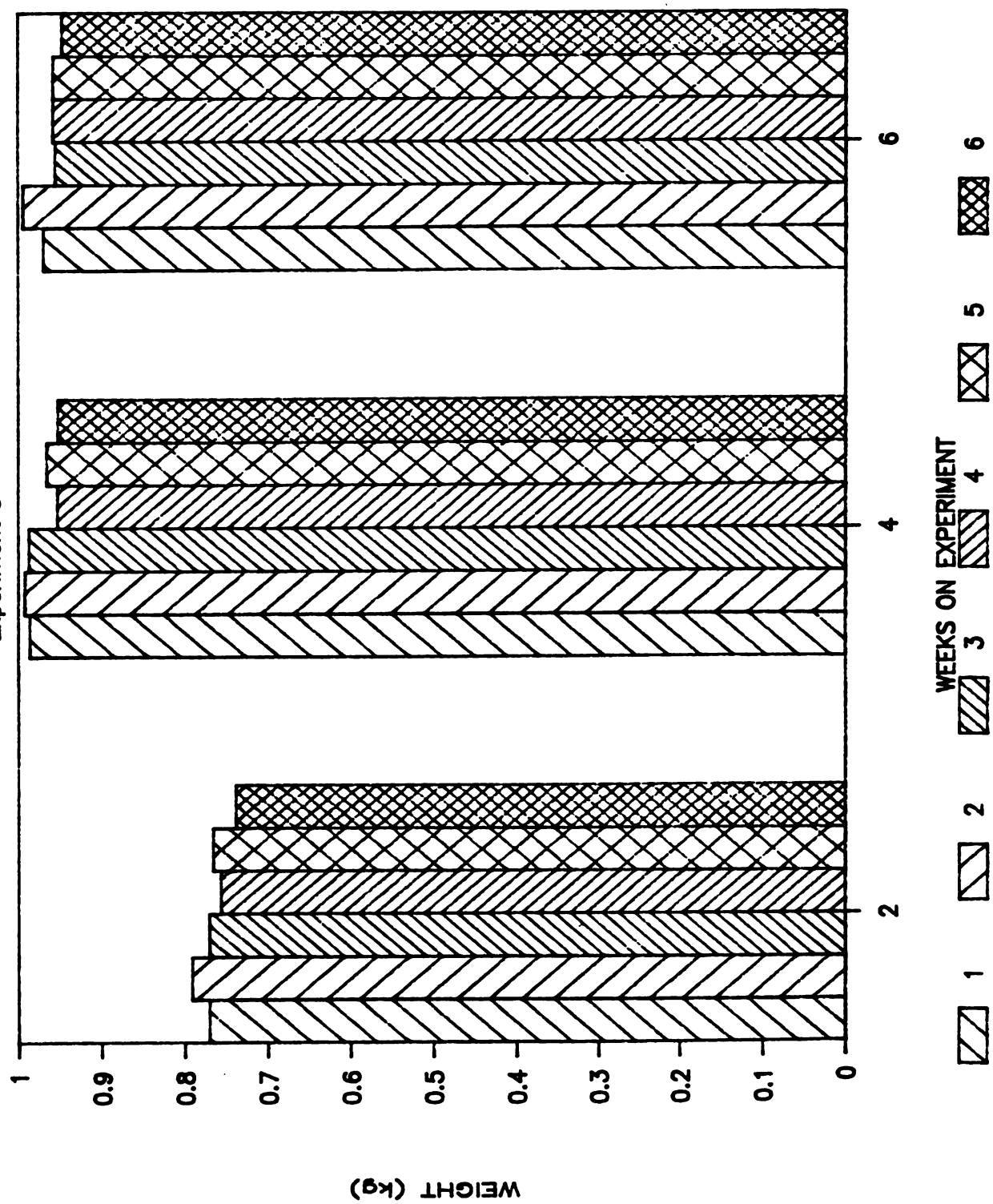


**Figure 2.** **The effects of calcium at .6, .7 or .8% of the dietary dry matter and a calcium to phosphorus ratio of 1.3:1 or 1:1.3, over time, on the body lengths of growing ferrets. Treatment effects NS (SEM .89). (Experiment 3)**

# FIGURE 2. BODY LENGTH

Experiment 3

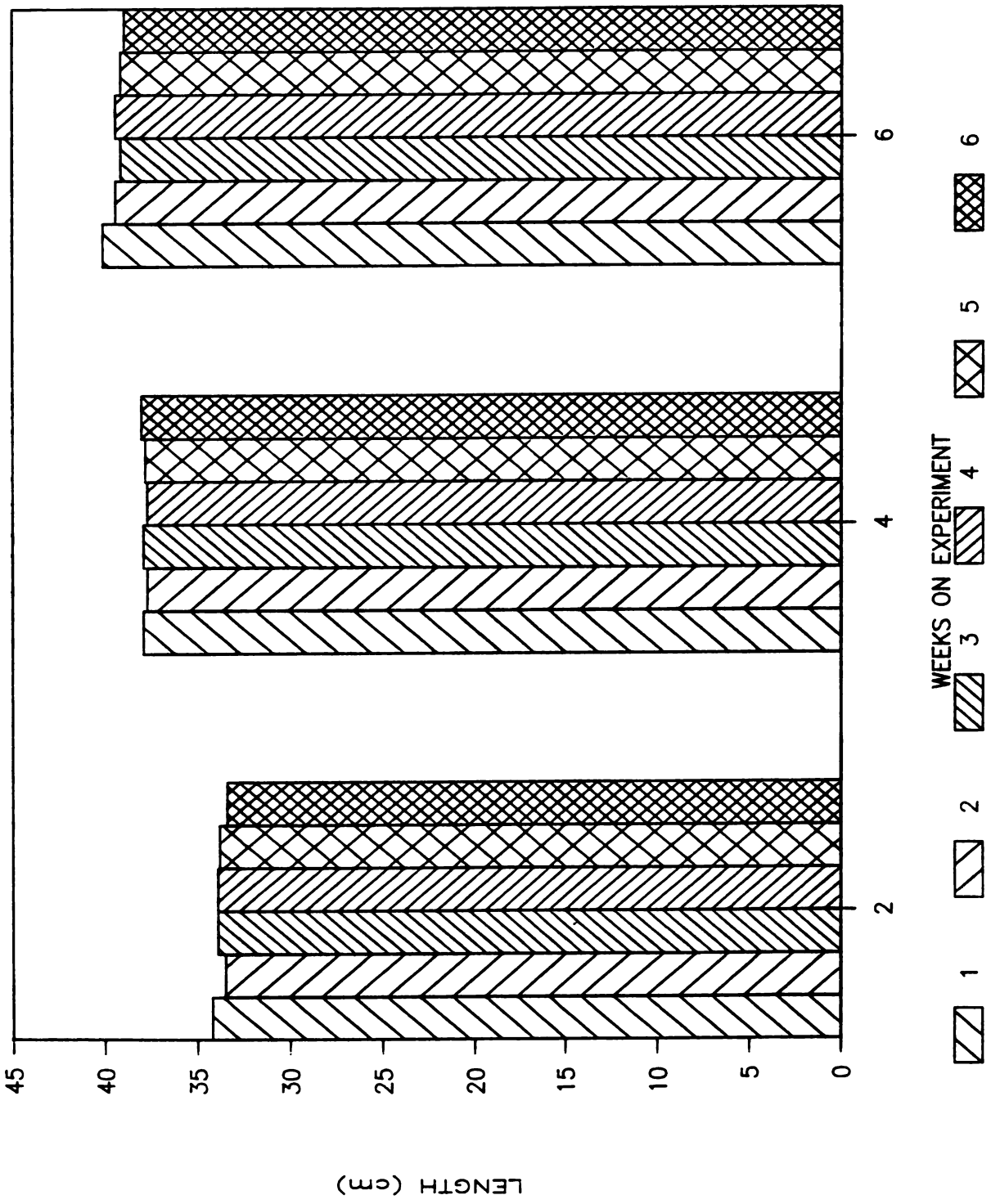


Table 3. Growth of Ferrets Fed Various Levels of Calcium and Resultant Calcium to Phosphorus Ratios[a] (Experiment 3)

| | Dietary calcium (% of dry matter) | | | | | |
|---|---|---|---|---|---|---|
| | .6 | | .7 | | .8 | |
| | Ca:P ratio | | | | | |
| | 1.3:1 | 1:1.3 | 1.3:1 | 1:1.3 | 1.3:1 | 1:1.3 |
| Body weight (g) | | | | | | |
| Initial | 557 | 564 | 575 | 542 | 568 | 526 |
| 1 Week | 662 | 673 | 663 | 638 | 661 | 634 |
| 2 Weeks | 770 | 791 | 770 | 756 | 766 | 738 |
| 3 Weeks | 871 | 886 | 861 | 850 | 875 | 848 |
| 4 Weeks | 986 | 992 | 987 | 953 | 966 | 952 |
| 5 Weeks | 1056 | 1061 | 1063 | 1047 | 1044 | 1026 |
| 6 Weeks | 969 | 994 | 955 | 958 | 958 | 947 |
| Net gain | 412 | 430 | 380 | 416 | 391 | 421 |
| Body length (cm) | | | | | | |
| Initial | 29.6 | 29.4 | 29.2 | 28.5 | 28.8 | 28.1 |
| 1 Week | 33.1 | 32.6 | 33.1 | 32.9 | 32.6 | 32.7 |
| 2 Weeks | 34.2 | 33.5 | 33.9 | 33.9 | 33.8 | 33.4 |
| 3 Weeks | 36.4 | 36.1 | 36.8 | 36.6 | 36.5 | 36.0 |
| 4 Weeks | 37.9 | 37.7 | 37.9 | 37.7 | 37.8 | 38.0 |
| 5 Weeks | 38.4 | 38.5 | 38.4 | 38.4 | 38.2 | 38.5 |
| 6 Weeks | 40.1 | 39.4 | 39.1 | 39.4 | 39.1 | 38.9 |
| Net increase | 10.5 | 10.0 | 9.9 | 10.9 | 10.3 | 10.8 |

[a]Ten observations per mean.

adequately regardless of Ca or P concentrations or the resultant Ca:P ratio in the diet. Although not statistically different, the ferrets consuming .7% Ca with a Ca:P ratio of 1.3:1 (Treatment 3), had the lowest net body wt gain and body length increase. Those groups with the greatest net body wt increase did not necessarily have the greatest net body length increase.

B. Plasma

There were significant (P <.001) effects of dietary Ca, Ca:P ratio and the Ca x ratio interaction on plasma Ca concentrations. The reason for the relative hypercalcemia on the lowest dietary Ca level is unclear (Table 4). Miller et al. (1962) reported increasing serum Ca concentrations with increasing dietary Ca levels from .6 to 1.2%. Dietary Ca:P ratios of 1.3:1 produced a higher (P <.001) plasma Ca concentration than a ratio of 1:1.3 (Table 5). The decline in plasma Ca may be due to the increased P content in the diets with a Ca:P ratio of 1:1.3 (Ushakov et al., 1983). Treatment 1 (.6% Ca, 1.3:1 Ca:P) produced a higher (P < .05) plasma Ca concentration than the other diets (Table 6). The hypercalcemia observed may be the result of the relatively low P intake (.46% of the dietary dry matter)as suggested by Cuisinier-Gleizes et al. (1975) and demonstrated in pigs by Miller and co-workers (1964).

Hoover and Baldwin (1987) reported serum Ca concentrations of ferrets 12 wk and 18 wk old to be in the ranges of 8.2 to 12.1 mg/dl and 9.0 to 10.3 mg/dl,

respectively. At the conclusion of this trial, ferrets were 14 wk of age and exhibited plasma Ca concentration comparable to the 18 wk old ferrets.

Dietary Ca level had a significant ($P < .01$) effect on plasma inorganic P (Table 4). As dietary Ca increased, so did plasma inorganic P concentrations. The Ca:P ratio also had a significant ($P < .001$) effect on plasma inorganic P concentrations (Table 5). Over all the treatments (Table 6), Treatment 2 with Ca at .6% and a Ca:P ratio of 1:1.3 produced the lowest plasma inorganic P concentration. All ferrets had plasma inorganic P concentrations in the range of 7.5 to 10.5 mg/dl which was reported as normal for ferrets 12 wk of age (Hoover and Baldwin, 1987).

There were slightly significant ($P < .10$) effects of the dietary Ca level and the Ca:P ratio on plasma creatinine concentrations (Tables 4 and 5). Values observed were greater than those reported for either 12 or 18 wk old ferrets, and these values increased with age (Hoover and Baldwin, 1987). The interaction between dietary Ca and Ca:P ratio had a significant ($P < .01$) effect on plasma creatinine. Ferrets consuming Treatment 6 (.8% Ca and 1:1.3 ratio of Ca:P) had a lower ($P < .01$) plasma creatinine concentration which was also most nearly the normal values of $.72 \pm .11$ mg/dl reported by Hoover and Baldwin (1987).

Plasma ALP activity was not affected by dietary Ca, Ca:P ratio or the interaction between the two. All groups were within ranges reported previously by Hoover and Baldwin

Table 4. Effects of Various Levels of Dietary Calcium on Plasma Concentrations of Calcium, Inorganic Phosphorus and Creatinine, and Alkaline Phosphatase Activities of Young Ferrets[a] (Experiment 3)

| | Dietary Ca (% of dry matter) | | | | |
|---|---|---|---|---|---|
| | .6 | .7 | .8 | SEM | P< |
| Ca (mg/dl) | 10.6[e] | 9.4[cf] | 9.7[df] | .09 | .001 |
| Inorg. P (mg/dl) | 7.3[e] | 7.5 | 7.8[f] | .10 | .001 |
| Creatinine (mg/dl) | 1.00 | 1.02[c] | .93[d] | .032 | .10 |
| ALP (U/L)[b] | 59.0 | 56.6 | 55.8 | 1.90 | NS |

[a]Twenty observations per mean.
[b]One unit defined as that amount of enzyme which will produce 1 micromole of p-nitrophenol per min under the conditions of Sigma assay procedure #245.
[cd]Means within a row with different letter superscripts are significantly different (P <.05).
[ef]Means within a row with different letter superscripts are significantly different (P <.01).

Table 5. Effects of Dietary Calcium to Phosphorus Ratios on Plasma Concentrations of Calcium, Inorganic Phosphorus and Creatinine, and Alkaline Phosphatase Activities of Young Ferrrets[a] (Experiment 3)

| | Dietary Ca:P ratio | | | |
|---|---|---|---|---|
| | 1.3:1 | 1:1.3 | SEM | P< |
| Ca (mg/dl) | 10.2 | 9.6 | .07 | .001 |
| Inorg. P (mg/dl) | 7.8 | 7.2 | .08 | .001 |
| Creatinine (mg/dl) | 1.02 | .95 | .03 | .10 |
| ALP (U/L)[b] | 57.2 | 57.0 | 1.56 | NS |

[a]Thirty observations per mean.
[b]One unit defined as that amount of enzyme which will produce 1 micromole of p-nitrophenol per min under the conditions of Sigma assay procedure #245.

Table 6. Effects of Various Levels of Dietary Calcium and Resultant Calcium to Phosphorus Ratios on Plasma Concentrations of Calcium, Phosphorus and Creatinine, and Alkaline Phosphatase Activities of Young Ferrets[a] (Experiment 3)

| | Dietary Ca (% of dry matter) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | .6 | | .7 | | .8 | | | |
| | Ca:P ratio | | | | | | | |
| | 1.3:1 | 1:1.3 | 1.3:1 | 1:1.3 | 1.3:1 | 1:1.3 | SEM | P< |
| Ca (mg/dl) | 11.2[g] | 9.9[eh] | 9.5[fh] | 9.4[fh] | 9.9[eh] | 9.5[fh] | .13 | .001 |
| Inorg. P (mg/dl) | 7.9[eg] | 6.7[eh] | 7.4[fh] | 7.7[g] | 8.2[g] | 7.3[fh] | .14 | .001 |
| Creatinine (mg/dl) | .96[c] | 1.05[e] | 1.13[de] | .91[df] | .97[c] | .88[f] | .045 | .01 |
| ALP (U/L)[b] | 58.3 | 59.7 | 58.7 | 54.6 | 54.7 | 56.9 | 2.70 | NS |

[a]Ten observations per mean.

[b]One unit defined as that amount of enzyme which will produce 1 micromole of p-nitrophenol per min under the conditions of Sigma assay procedure #245.

[cd]Means within a row with different letter superscripts are significantly different (P <.05).

[ef]Means within a row with different letter superscripts are significantly different (P <.01)

[gh]Means within a row with different letter superscripts are significantly different (P <.001).

(1987) for young ferrets.

C. Femurs

Neither the dietary Ca, the Ca:P ratio nor their interaction had any effect on the fresh weight, total length or shaft length of the ferret femurs (Tables 7, 8 and 9). On a dry weight basis, however, there was a trend for Treatment 6 to produce the lightest femurs. This agrees with the findings of Draper et al. (1972) who reported that a decrease in femur wt occurred when the level of P exceeded that of Ca in the diet of aged rats. On the other hand, in young rats, femur wt increased when dietary P was raised from .35 to .8% (Howe and Beecher, 1983).

None of the dietary treatments had an effect on the A-P diameter of the ferret femurs. However, dietary Ca concentration had a significant ($P < .05$) effect on the L-M diameter (Table 7). L-M femur diameters decreased with increasing dietary Ca concentrations and the highest level of Ca (.8%) resulted in a smaller ($P < .05$) diameter than the lowest Ca level (.6%).

Maximum breaking force is that amount of force in Kilonewtons (KN) required to break the bone, and serves as an indicator of strength. There was no difference between treatment groups in the force required to break the femurs. Neither dietary Ca (Table 7) nor Ca:P ratio (Table 8) had an effect on the maximum breaking force sustained by the femurs.

Bending moment refers to the bone's flexibility or its ability to bend when stress is applied. Dietary Ca (Table 7)

Table 7. Effects of Various Levels of Dietary Calcium on Selected Physical and Chemical Measurements of Ferret Femurs[a] (Experiment 3)

| | Dietary Ca (% of dry matter) | | | | |
|---|---|---|---|---|---|
| | .6 | .7 | .8 | SEM | P< |
| Fresh weight (g) | 1.95 | 1.89 | 1.85 | .044 | NS |
| Total length (mm) | 49.8 | 50.1 | 49.7 | .38 | NS |
| Shaft length (mm) | 36.5 | 37.0 | 36.6 | .43 | NS |
| A-P diameter[b] (mm) | 3.5 | 3.7 | 3.5 | .09 | NS |
| L-M diameter[c] (mm) | 3.8 | 3.7 | 3.6 | .05 | .05 |
| Breaking force (KN) | .157 | .165 | .166 | .005 | NS |
| Bending moment (KN-mm) | 1.4 | 1.6 | 1.6 | .04 | .10 |
| Dry matter (%)[d] | 56.9[e] | 58.6[f] | 57.5 | .39 | .05 |
| Ash (%)[d] | 54.7[e] | 55.3[fg] | 54.6[h] | .17 | .05 |
| Ca in ash (%) | 33.7[e] | 33.1 | 32.8[f] | .29 | .10 |
| P in ash (%) | 15.6[g] | 15.8[g] | 18.4[h] | .10 | .001 |
| Mg in ash (%) | .63 | .60 | .63 | .011 | NS |
| Na in ash (%) | .96 | .98[g] | .94[h] | .010 | .05 |
| K in ash (%) | .42[g] | .32[h] | .40[g] | .015 | .001 |

[a]Twelve observations per mean.
[b]Anterior to posterior.
[c]Lateral to medial.
[d]Expressed on a dry, fat-free basis.
[ef]Means within a row with different letter superscripts are significantly different (P <.05).
[gh]Means within a row with different letter superscripts are significantly different (P <.01).

Table 8. Effects of Dietary Calcium to Phosphorus Ratios on Selected Physical and Chemical Measurements of Ferret Femurs[a] (Experiment 3)

| | Dietary Ca:P ratio | | | |
|---|---|---|---|---|
| | 1.3:1 | 1:1.3 | SEM | P< |
| Fresh weight (g) | 1.91 | 1.89 | .036 | NS |
| Total length (mm) | 49.9 | 49.9 | .31 | NS |
| Shaft length (mm) | 36.6 | 36.8 | .35 | NS |
| A-P diameter[b] (mm) | 3.6 | 3.5 | .07 | NS |
| L-M diameter[c] (mm) | 3.7 | 3.8 | .04 | NS |
| Breaking force (KN) | .166 | .160 | .0033 | NS |
| Bending moment (KN-mm) | 1.54 | 1.52 | .033 | NS |
| Dry matter (%)[d] | 58.1 | 57.2 | .32 | .10 |
| Ash (%)[d] | 54.8 | 54.9 | .14 | NS |
| Ca in ash (%) | 33.0 | 33.4 | .23 | NS |
| P in ash (%) | 16.7 | 16.5 | .08 | .05 |
| Mg in ash (%) | .63 | .62 | .009 | NS |
| Na in ash (%) | .96 | .95 | .007 | NS |
| K in ash (%) | .36 | .40 | .012 | .05 |

[a]Eighteen observations per mean.
[b]Anterior to posterior.
[c]Lateral to medial.
[d]Expressed on a dry, fat-free basis.

Table 9. Effects of Various Levels of Dietary Calcium and Resultant Calcium to Phosphorus Ratios on Selected Physical and Chemical Measurements of Ferret Femurs[a] (Experiment 3)

| | Dietary Ca (% of dry matter) | | | | | | |
|---|---|---|---|---|---|---|---|
| | .6 | | .7 | | .8 | | SEM |
| | Ca:P ratio | | | | | | |
| | 1.3:1 | 1:1.3 | 1.3:1 | 1:1.3 | 1.3:1 | 1:1.3 | |
| Fresh weight (g) | 1.96 | 1.94 | 1.86 | 1.92 | 1.99 | 1.81 | .061 |
| Total length (mm) | 49.8 | 49.8 | 49.9 | 50.4 | 49.9 | 49.5 | .53 |
| Shaft length (mm) | 36.4 | 36.6 | 36.6 | 37.4 | 36.9 | 36.3 | .61 |
| A-P diameter[b] (mm) | 3.6 | 3.5 | 3.8 | 3.6 | 3.5 | 3.4 | .13 |
| L-M diameter[c] (mm) | 3.9[e] | 3.8 | 3.8 | 3.8 | 3.6[f] | 3.7 | .07 |
| Break. force (KN) | .154 | .160 | .173 | .157 | .169 | .163 | .006 |
| Bend. mom. (KN-mm) | 1.39[eg] | 1.51 | 1.64[h] | 1.52 | 1.60[f] | 1.53 | .058 |
| Dry matter (%)[d] | 57.0[g] | 56.8[g] | 59.4[eh] | 57.8[f] | 58.0 | 57.1[g] | .55 |
| Ash (%)[d] | 54.7 | 54.8 | 55.3[e] | 55.3[e] | 54.5[f] | 54.7 | .24 |
| Ca in ash (%) | 33.7[e] | 33.7[g] | 33.2 | 33.0 | 32.2[fh] | 33.4[e] | .41 |
| P in ash (%) | 15.7[g] | 15.6[eg] | 16.0[fg] | 15.6[eg] | 18.5[h] | 18.3[h] | .15 |
| Mg in ash (%) | .63 | .64 | .60 | .61 | .66 | .61 | .159 |
| Na in ash (%) | .94[eg] | | .97[h] | .99[fh] | .97[h] | .97[h] | .92[g].013 |
| K in ash (%) | .43[g] | .41[g] | .27[h] | .38[g] | .38[g] | .42 | .022 |

[a]Six observations per mean.
[b]Anterior to posterior.
[c]Lateral to medial.
[d]Expressed on a dry, fat-free basis.
[ef]Means within a row with different letter superscripts are significantly different (P <.05).
[gh]Means within a row with different letter superscripts are significantly different (P <.01).

as well as the interaction between Ca and Ca:P ratio (Table 9) of the diet, had a marginally significant (P <.10) effect on femur bending moment (KN-mm). Femurs taken from animals consuming .6% dietary Ca (Treatment groups 1 and 2) had lower bending moments than those from animals consuming .7 or .8% Ca. Treatment group 1 (.6% Ca, Ca:P of 1.3:1) had the lowest overall bending moment of 1.39 KN-mm, whereas, Treatment group 3 (.7% Ca, Ca:P of 1.3:1) had the highest bending moment of 1.64 KN-mm.

There was a significant (P <.05) effect of dietary Ca on femur dry matter (Table 7), in that the lowest Ca level (.6%) resulted in the lowest percent dry matter. Dietary Ca at .7% resulted in the greatest femur dry matter and also the greatest (P <.05) femur ash percent (Table 7). There were no significant effects of dietary Ca:P ratio or the interaction between Ca and ratio on femur ash.

Ca concentration of the diet had a marginally significant effect (P <.10) on femur Ca (% in ash). Ca at .6% resulted in the highest Ca percent in femur ash (Table 7). The Ca:P ratio and the interaction between dietary Ca and Ca:P ratio had no effects on femur Ca concentration.

Femur P in the ash was affected significantly by both the Ca level in the diet (P <.001) and the Ca:P ratio of the diet (P <.05). The interaction between the two was not significant. Dietary Ca at .8% produced a higher (P <.001) percent P in the ash than either .6 or .7% dietary Ca. A Ca:P ratio of 1.3:1 resulted in a higher percent P in the

femur ash than the 1:1.3 ratio. This, however, was a small difference that probably was not physiologically significant.

Howe and Beecher (1983) suggested that excessive P concentration in bone may impede flexibility and that high P intakes could be detrimental to bone flexibility during rapid growth. In this trial, however, high dietary P was not associated with a lower bending moment.

There were no treatment effects on femur ash Mg concentrations. Dietary Ca had a significant ($P < .05$) effect on the Na percent in the ash (Table 7). Ferrets consuming the high level of dietary Ca (.8%) had femurs with the lowest Na concentrations in the ash. The Ca and Ca:P ratio interaction also had a significant ($P < .01$) effect on femur ash Na concentration (Table 9). When comparing all the treatments, Treatment 6, which not only had .8% Ca but the inverse Ca:P ratio of 1:1.3, produced the lowest femur Na concentration.

The Ca concentration of the diet had a significant ($P < .001$) effect on femur ash K concentration (Table 7). In this instance, the middle level (.7%) of Ca produced the lowest femur K. The dietary Ca:P ratio had a lesser ($P < .05$) effect on femur K concentration. Treatments with a ratio of 1:1.3 produced femurs with a higher percent K in the ash (Table 8). It follows that consumption of Treatment 3 (.7% Ca, 1.3:1) resulted in the lowest femur K concentration of all the test diets (Table 9).

An inverse Ca:P ratio in the diets fed to young ferrets had no adverse effects on their growth and development for

this short-term period. This ratio is at the lower end of the range recommended by the NRC (1982) for growing mink.

Since Treatment 1 resulted in a relatively higher plasma Ca concentration, this finding suggests that .46% dietary P may be too low to maintain normocalcemia.

Feeding acidic substances in the diets of animals has been shown to result in bone resorption (Petito and Evans, 1984). However, in this trial, ferrets consuming high concentrations of $H_3PO_4$ exhibited no marked differences in femur mineral concentrations. Although statistical differences due to treatment were found for some of the physical and chemical measures of the femurs, none were so large to suggest any physiological complications.

The exact nature of the interactions between dietary Ca, P and their ratio on the growth and development of the ferret cannot be delineated from these data alone. Results from this experiment, nonetheless, suggest that the ferret is capable of maintaining normal growth as well as those measured plasma and bone constituents on diets with an inverse Ca:P ratio of 1:1.3 when dietary Ca is supplied at .6,.7 or .8%. The growing ferret also exhibited no adverse reactions to the $H_3PO_4$ in the diet and appeared to be able to utilize the P supplied by it.

# GENERAL SUMMARY AND CONCLUSIONS

The cat and mink are mammalian carnivores and are often used as representatives of such in a research setting. Nutritionally, the feline has been the most extensively studied domestic mammalian carnivore. The cat requires a relatively high level of dietary protein and also requires a dietary source of niacin, arachidonic acid, taurine, arginine and preformed vitamin A. All of these so-called "nutritional idiosyncrasies" of the cat are believed to be evolutionary adaptations associated with strict adherence to a carnivorous diet. The mink and ferret share some of these peculiar nutritional requirements with the cat.

The cat's quantitative requirements for essential minerals do not greatly exceed those of the pig or rat, and the cat does not have a requirement for any mineral that is not essential for these species. However, there still exists the possibility of peculiarities in the transport, metabolism, or storage of minerals by cats and other carnivores (MacDonald et al., 1984). The relatively low Ca and high P concentrations present in skeletal muscle suggest that the carnivore may also have evolved with an ability to tolerate a dietary Ca:P ratio of less than one.

Another similarity between the cat and the two mustelids is the susceptibility to struvite ($MgNH_4PO_4.6H_2O$) urolithiasis. Since the solubility of struvite uroliths increases with a decreasing pH of the urine, urinary

acidification is not only a method of treatment for urolithiasis, but may also be employed as a preventive measure.

In studies reported in this thesis, a diet composed primarily of meat, plus the addition of $H_3PO_4$ as a urinary acidifier, provided the ferret with relatively high levels of both protein and P. The ferret was able to adapt to the presumed excess of dietary P by increasing P excretion in the urine.

Because P is a component of struvite, the increased P concentration in the urine would be expected to increase the ferret's susceptibility to struvite urolith formation. However, the coincident decrease of urine pH to less than 6.4, resulting from the dietary $H_3PO_4$, would be sufficient to prevent struvite precipitation.

Relatively high levels of dietary P and the resultant inverse Ca:P ratios did not affect Ca and P concentrations or proportions in the plasma of the ferret. In these short term trials, $H_3PO_4$ consumption did not result in metabolic acidosis. Marked hypercalciuria or hypocalcemia were not observed in the ferrets consuming diets with high levels of P and/or an inverse Ca:P ratio. The high P concentrations in the diets most often produced a decrease in urinary Ca concentrations. Ferrets found the diets with $H_3PO_4$ additions palatable, and no feed refusal or vomiting was noted, as sometimes occurs when $NH_4Cl$ is fed as a urinary acidifier to cats.

In the young ferret, no adverse effects of dietary $H_3PO_4$

upon growth or bone development were observed in this short term. Femurs examined were similar in size, strength, flexibility and mineral concentration whether the dietary Ca:P ratio was 1.3:1 or 1:1.3. When the dietary Ca concentration was .6, .7 or .8% of the dry matter, the growing ferret not only tolerated, but thrived on the inverse dietary Ca:P ratio.

Phosphoric acid is concluded to be an effective urinary acidifier which did not have detrimental effects on the Ca and P homeostasis of either adult or young ferrets. Data from these experiments suggest that the optimal level of $H_3PO_4$ (85%) added to the diet for maximum urinary acidification lies between 1.86 and 2.98% of the dietary dry matter. Adult ferrets were capable of adapting to a Ca:P ratio as low as 1:4 when adequate dietary Ca was provided. Growing ferrets tolerated dietary Ca:P ratios of 1:1.3 when Ca concentration in the diet was at least .6%.

Results from these short term studies are indicative of the ferret's capabilitity of adapting to a diet which is relatively high in P and contains an inverse Ca:P ratio. Further studies are necessary to elucidate the long term effects of the diets described above. If high levels of dietary P and an inverse dietary Ca:P ratio can be demonstrated to support the life stages of the ferret, perhaps this phenomenon may also be considered an evolutionary adaptation, unique to the mammalian carnivore.

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