EFFICACY OF AMMONIUM CHLORIDE AND HORSE GRAM *(Dolichos biflorus)* EXTRACT ON THE AMELIORATION OF UROLITHIASIS IN GOATS

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled "**EFFICACY OF AMMONIUM CHLORIDE AND HORSE GRAM (*Dolichos biflorus*) EXTRACT ON THE AMELIORATION OF UROLITHIASIS IN GOATS**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

Introduction

1. INTRODUCTION

Urolithiasis is a well-known pathological condition of urinary tract met within human and livestock including poultry. Urinary calculi are commonly observed at necropsy in normal animals with little or no harm. The important effect of urolithiasis is in the production of urethral obstruction. The understanding of simple urolithiasis and obstructive urolithiasis is important. Simple urolithiasis is relatively little importance, but obstructive urolithiasis is a fatal condition, if the obstruction is not relieved. Rupture of the urethra or bladder occurs and the animal dies of uremia or due to secondary bacterial infection, causing great economic loss to livestock industry. The simple uroliths, at any time when sufficiently large size cause obstruction in the passage of urine.

Rupture of urethra is more common with irregularly shaped stones which cause partial obstruction and pressure necrosis of the urethral wall.

Although the occurrence of obstructive urolithiasis is sporadic, cases occur at irregular intervals in groups of animals. Outbreaks have been recorded in which large number of animals are affected in short time (Udall and Jensen, 1958).

A systematic study on the incidence of urolithiasis in ruminants is lacking in India. However, available literature and reports from various regions indicated that, large number of cattle and buffaloes are suffering from urolithiasis. Published reports concerning the incidence in ruminants in India are several (Jayakumar, 1991; Kumar *et al.*, 1991; Ashturkar, 1992; Khanna and Puranchand, 1993; Ashturkar, 1994; Mohinder Singh *et al.*, 1995 and Harnam Singh and Sahu, 1995). Incidences have been reported to be high in the states of Punjab, Haryana, Rajasthan, Uttar Pradesh, Bihar, Gujarat, Madhya Pradesh, Orissa, Andhra Pradesh and Tamil Nadu (Jit Singh and Kuldip Singh, 1990). Most of these studies were based on the figures obtained from cases admitted to institute clinics or from slaughter houses.

Incidence of urolithiasis occur equally in both sexes, but obstruction is commonly seen in male because of long, narrow urethra.

Urolithiasis in ruminants composed mainly of either phosphates or silica associated with a protein matrix. Silica uroliths are almost found in range animals whereas, uroliths composed of phosphatic salts occur principally in feed lot animals consuming high concentrates, composed mainly of cereals. Less frequent type of uroliths include carbonate and oxalate (Hawkins, 1965; Larson, 1996).

Since the obstructive urolithiasis is a fatal condition, treatment mainly involves surgical removal of calculi, but the condition has more chance to recur. When condition favouring urolithiasis is encountered prevention is ofcourse preferable.

The incidence of urolithiasis in man and animals are reported frequently and any effort to stop its advancement is worthwhile. With regard to amelioration and prophylactic measures of urolithiasis in livestock, very limited work has been done and several chemical agents have been tried in India and in abroad and some of the indigenous plant-agents have been recognised for its anticalculogenic activity. From a perusal of the literature on the incidence, magnitude of prevalence of urinary calculi and its possible prevention, it is seen that hardly any information is available on these aspects as applied to goats.

Hence, it is an imperative need to sort out chemical and plant agents which have ameliorating effect and can be used as therapeutic agents for the prevention and treatment of urinary calculi in ruminants, especially in goats.

The investigation proposed to be carried out here are confined mainly to probe in depth whether some of the chemical and plant agents can prevent the incipience of uriolithiasis in goats. This problem has been put forward after careful

consideration of reported anticalculogenic properties of some chemical and plant agents and some preliminary studies on the same (James, 1968 and 1973; James and Mukundan, 1978).

Hence the work proposed is highly relevant with regard to scientific advancement in this aspect as well as overcoming the problem of urinary calculi in ruminants, especially in goats.

Review of Literature

2. REVIEW OF LITERATURE

This study involves induction of urolithiasis with a dietary factor known to cause calculi (James, 1973) and its prevention with chemical and plant agents like ammonium chloride and horse gram extract, known to have anticalculogenic activity (Das, 1956; Bushman *et al.*, 1967, 68; Crookshank, 1970; Yano and Kawashima, 1977 and Archana and Singla, 1994). The research work carried out on the above aspects as applied to goats is rather scanty. A brief review of most recent and relevant literature on the above aspects are presented here.

2.1 Etiology of urolithiasis

Many predisposing, contributing and causative factors are responsible for urolith formation and development. But the pathogenesis of urolith formation may not be the same in all cases and sometimes may involve more than one factor.

Vitamin A deficiency has been suggested as a possible inciting factor in the formation of silicious calculi by Swingle and Marsh (1956).

Dutt *et al.* (1959) observed that goats deprived of Vitamin A manifested widespread keratinisation of tubular epithelium, some tubules of the renal pelvis showing shed

epithelial cells. In such condition there is sufficient irritation to produce fibrin and mucin which forms frame work for the deposition of sediment and subsequent formation of stones.

Marsh (1961) recorded 10 per cent incidence of obstructive urolithiasis in feedlot lambs receiving a supplement of stilbesterol at the rate of one milligram per kilogram of feed or two milligram per lamb daily.

Crookshank *et al.* (1965) found that lambs maintained on pelleted feed had more calculi, excreted less magnesium and potassium and more phosphorus through urine than lambs given the same diet as meal.

Packett and Coburn (1965) observed that supplementation of Vitamin D at the rate of 440 IU per kilogram of feed to sheep maintained on pelleted ration cause increased occurrence of urolithiasis.

Ranjan *et al.* (1965) reported the incidence of nephrocalcinosis in buffaloe calves due to drinking of hard water.

Udall and Chow (1965) observed that lambs fed with high proportion of concentrate, cause increased excretion of

protein and bound hexosamine through urine that favours calculi formation.

Hosseinion *et al.* (1976) reported that urolithiasis in a flock of sheep was due to low calcium to phosphorus ratio and Vitamin A deficiency.

McIntosh *et al.* (1974) analysed mineralogical composition of uroliths occurred in field outbreak, where in 47 per cent had mixture of calcium oxalate and silica, 75 per cent contained at least either one of these compounds the reason due to high level of silica content of native pasture.

Nottle (1976) reported that sheep fed with estrogenic clover developed urinary sediments.

Bailey (1976) reported that low water turnover, indicating low water intake, leading to a high concentration of silica in urine of range animals which is necessary, but not a sufficient condition for silica calculi formation.

Both Linklater and Angus (1979) and Waltner-towes and Meadows (1980) observed in feeder rams and cattle, a very high incidence of urinary calculi and they found that oxalate in the feed contaminated with oxalate-producing fungi as the causative factor.

Bhaskar Singh *et al.* (1985) also suggested that feeding of high oxalate containing paddy straw and legumes contribute major source of oxalate, related to oxalate calculi in buffaloes.

Blood and Radostits (1989) opined that precipitation of solutes due to concentration of urine consequent on continued deprivation of water is often exacerbated by heavy fluid loss by sweating in hot, arid climates.

Hypervitaminosis D has been suggested as a cause for excess urine concentration of calcium especially in hot climate where dehydration may also be a contributing factor (Blood and Radostits, 1989).

Stewart *et al.* (1990 and 1991) reported that lambs fed freely with 50 per cent hay and ground oat diet (with Ca:P of 1:1) with silica oxide at the rate of 3.4 per cent alone or in combination with supplemention of 0.75 per cent limestone (with Ca:P of 2:1) developed silicious kidney deposits and suggested that high silica diet having high calcium to phosphorus ratio and alkali forming potential contributed to silica urolithiasis.

2.2 Role of minerals in the formation of urinary calculi

2.2.1 Calcium and phosphorus

Crookshank and Robbins (1962) observed that those sheep excrete more phosphorus through urine later developed urinary calculi, but no calculi in lambs with low phosphorus excretion through urine and this might forecast whether the diet would be likely to cause calculi in sheep. Similar observations were reported by Packett *et al.* (1968), Mayer (1972) and Godwin and William (1982).

Emerick and Embry (1963) investigated the role of calcium and phosphorus related to development of urinary calculi in lambs in which they observed that a dietary supplementation of 0.62 per cent phosphorus and 0.44 per cent calcium, 31 per cent of lambs had urinary calculi and that proportion has been increased to the extent of 73 per cent with 0.81 per cent dietary phosphorus.

Bushman *et al.* (1965) observed in wether lambs that higher level of dietary phosphorus resulted in higher level of serum and urine phosphorus and urinary calculi incidence. They suggested calcium to phosphorus ratio of 2:1 in the ration appeared to be optimum.

Robbins *et al.* (1965) reported that increased intake of phosphorus tend to produce calculi of magnesium phosphate type in lambs. He also observed that, with high dietary phosphorus and low calcium there was more phosphorus excretion through urine with increased incidence of urinary calculi.

Cottereau *et al.* (1966) reported high incidence of urolithiasis in adult male sheep flock fed with a ration having calcium to phosphorus ratio of 3:1 and 7:1. They also had access to salt lick with more calcium than phosphorus and drinking water coming from calcarious regions.

Hoar *et al.* (1970a) indicated that high phosphorus and low calcium in the diet tend to produce calculi, which have its size more with higher level of potassium in the diet.

Bellanger *et al.* (1981) reported the occurrence of urolithiasis in adult goats when fed with a ration having 2750 µg/g of calcium 2500 µg/g of magnesium and 41000 µg/g of phosphorus. The main symptom of obstruction was dysuria.

Gerald *et al.* (1984) observed that hydroxyproline, an oxalate precursor in serum was found to be more in steers fed with low calcium (0.3 per cent), a factor responsible for oxalate calculi in steers.

Koutinas *et al.* (1989) reported an outbreak of urolithiasis in fattening male lambs fed with diet having 1.03 per cent calcium, 0.65 per cent phosphorus and 0.53 per cent magnesium per kilogram of dry matter and suggested high phosphorus and magnesium in the diet were the causative factors involved.

Ahmed *et al.* (1990) reported phosphatic urolithiasis in Egyptian calves, fed with high concentrate ration providing more phosphorus than calcium.

2.2.2 Magnesium

Crookshank *et al.* (1967) reported that lambs fed with high magnesium diet later developed urolithiasis. They observed a significant positive relationship between serum magnesium and the proportion of lambs with calculi, but no significant relationship with either calcium or phosphorus concentration in serum. Serum phosphorus and magnesium concentrations were more and serum calcium concentration was less in lambs developed urolithiasis, which will also forecast increasing susceptibility to urolithiasis.

James (1968) observed that a ration having 0.386 per cent calcium 0.267 per cent phosphorus and 0.6 per cent magnesium produced only crystalluria in goats over 10 weeks feeding trial, indicating that goats need and tolerate fairly good

amount of calcium, phosphorus and magnesium. In another investigation (James, 1973), goats maintained on ration containing 1.220 per cent calcium, 0.628 per cent phosphorus and 1.102 per cent magnesium induced urolithiasis of magnesium ammonium phosphate type.

Jones and Dawson (1976) found that a ration having 0.56 per cent calcium, 0.63 per cent phosphorus and 0.59 per cent magnesium (Ca:P:Mg = 1:1.12:1.05) was calculogenic to lambs because of high phosphorus and magnesium.

James and Mukundan (1978) observed that when jack leaves (*Artocarpus heterophyllus*) alone forms a major part of goat's ration, there will be incidence of urolithiasis and found that high magnesium content (0.7 to 0.8 per cent) in jack leaves was the causative factor involved. They also suggested that a ratio of concentrate to jack leaves 1:1 appeared satisfactory for proper absorption and utilization of calcium, phosphorus and magnesium.

Rice and McMurray (1981) investigated outbreaks of obstructive urolithiasis in calves fed with *ad libitum* concentrate containing 4.9 to 9.2 gram magnesium per kilogram of dry matter which was found to be the causative factor. The calculi mainly composed of magnesium ammonium phosphate.

Orskov and Robinson (1981) suggested that in fattening male lambs, the major causative factor for urolithiasis was too high inclusion rate of magnesium in the ration, since further incidence had prevented by lowering the supplementation of magnesium.

Kallfelz *et al.* (1986) found that increased dietary magnesium (1.4 per cent) alone or in combination with high phosphorus (1.6 per cent) induced urinary calculi in calves. Affected animals had three times the normal value of serum magnesium and significant depression in blood calcium. The calculi mainly composed of calcium apatite. This effect of high magnesium diet was somewhat overcome by addition of calcium (1.8 per cent) to the diet.

Cuddeford (1987) stated that high dietary level of magnesium (>2 g/kg DM) has been implicated as the main causal factor of urinary calculi in concentrate fed lambs.

Poole (1990) reported increasing incidence of urolithiasis in lambs when fed with 0.35 to 0.65 per cent of magnesium, but not with 0.12 and 0.27 per cent of magnesium and increasing the phosphorus level from 0.3 to 0.5 per cent in the diet did not affect the incidence.

2.3 Mechanism of calculi formation

Exact mode of calculogenesis is speculative. Induction however, involves several interrelated complex physiological and pathological factors. In general occurrence of the disease attributed to three theories, viz., crystalization inhibition theory, matrix nucleation theory and precipitation-crystalization theory (Jubb *et al.*, 1991).

The first concept is crystalization-inhibition theory of urolith initiation. Ultramicroscopical studies of urine of sheep and cattle by Puntriano (1954) showed that in both species there is a complex colloids and crystalloids held dispersed in a fluid phase. In healthy subjects most of the crystalloid structures are coated by accumulation of protective colloid material which presumably prevent the growth of crystals. Another one, hydrophobic colloid has the property of aggregating into clumps in the presence of electrolytes (crystalloids). Inadequate concentration of protective colloids enhance the clumping tendency of hydrophobic colloids thus leading to opposite of protection called "sensitization" cause crystal growth and calculi formation. It is postulated that colloid-crystalloid balance may be influenced by conditions such as mineral and fluid intake and by environmental stress.

Robertson *et al.* (1977) observed in humans that idiopathic calcium oxalate stone former's urine were more supersaturated with calcium oxalate and had low concentration of protective inhibitors of crystallization than those of normal subjects.

According to matrix nucleation theory, organic matrix (mucoproteins) form a nucleus and precipitation of crystalloids occur around it lead to the formation of stones. A `nidus' or organic nucleus plays an important role in the formation of pathological concretions. A nidus, usually in the form of group of desquamated epithelial cells or necrotic tissue favours the deposition of crystals by itself (Blood and Radostits, 1989).

Both Vitamin A deficiency and high intake of estrogen cause sloughing of tubular epithelial cells which forms matrix for crystal growth (Dutt *et al.*, 1959, Udall and Chow, 1965 and Nottle, 1976).

Danowski (1962) indicated that injury to connective tissue release mucoprotein which serves as a matrix for *in situ* calcification and renal calculus formation.

Romanowski (1965) mentioned that low molecular weight electrolytes in the urine release mucoprotein due to tissue

injury, which play an important role in providing matrix for calculi formation.

Phenylbutazone therapy (Read, 1983) or infection (Divers *et al.*, 1989) cause increased proteinacious inflammatory products or necrotic tissues, may serves as a matrix for precipitation of crystalloids.

The third concept of urolith formation is precipitation-crystalization theory. Urine is often supersaturated with respect to the component of stone-forming salts and this supersaturation is an essential precursor to the initiation of urolith formation (nucleation). Precipitation of urinary crystalloids occur, when the limit of supersaturation exceeded because of increased concentration of crystalloids or may be due to insufficient urine volume or hyper excretion of crystalloids (Jubb *et al.*, 1991).

Kathleen Lonsdale (1968) indicated that all stone forming compounds are insoluble, but solubility of stone forming compounds increases markedly with increase in temperature, change of pH or change in chemical composition of solvent. The passing of sandy urine, however, should be taken as danger signal because, epitaxial deposit will occur. The radial layer in cross sections of many stones almost certainly correspond with diurnal or seasonal variation in pH and less certainly with urine temperature.

Struvite uroliths found frequently in urinary tract of dog and humans due to infection in urinary tract which increases the pH of urine from acidic to alkaline, play an important role in precipitation of crystalloids (Osborne *et al.*, 1981).

Blood and Radostits (1989) mentioned that urine stasis gives more time for solute to deposit and calculi development.

Jit Singh and Kuldip Singh (1990) explained that the pH of urine affects solubility of some solutes altering solubility of urinary colloids. They also opined that mixed phosphate and carbonate calculi are more readily formed in an alkaline medium whereas in an acidic medium, silica calculi are formed. The pH of urine of an animal determine the salt that are to be precipitated. At pH below 5.0 uric acid, at 5.0 to 6.5 oxalate and at above 7.0 phosphate will be precipitated.

2.4 Amelioration and Prophylactic measures of urolithiasis

The effective prophylaxis of urinary calculi is the identification and removal of etiological factors involved. Since formation of a calculi is not dependent upon one factor, but interaction of number of factors, it is difficult to lay down definite recommendations to prevent the disease in areas

of varied geographical locations. Any method devised should also be simple to apply. Some recommendations have been made based on the results of experimental and clinical work.

2.4.1 Calcium

Both Emerick and Embry (1963) and Hoar *et al.* (1970a) reported that the incidence of phosphatic urolithiasis in sheep fed with a ration having low calcium to phosphorus ratio, was reduced by raising the calcium level above the level of phosphorus in the ration.

Bushman *et al.* (1967,1968) reported that raising the calcium level in the ration with calcium chloride and calcium carbonate at the rate of 1.5 to 2 per cent of the diet had reduced the incidence of phosphatic urolithiasis in lambs.

Vipperman *et al.* (1969) stated that calculogenic effect of high grain diet (0.3 per cent phosphorus and 0.16 to 0.19 per cent calcium) can be alleviated by addition of calcium carbonate. With these rations a calcium to phosphorus ratio of 1.3:1 is suboptimal but a ratio of 1.5:1 or 2:1 is more desirable.

Hoar *et al.* (1970b) also reported that incidence of phosphatic calculi with 0.47 per cent phosphorus and 0.31 per cent calcium was reduced by increasing the level of

calcium to 1.06 per cent with ground limestone, but completely prevented by adding calcium chloride at the rate of 0.56 per cent to the ration.

Yano *et al.* (1977) indicated the efficacy of calcium chloride in preventing calculi formation in wethers fed with high phosphorus and low calcium diet may be due to lowered phosphorus excretion through urine.

Godwin and Williams (1982) reported that supplementation of calcium carbonate to high grain diet reduced the urinary excretion of phosphorus without much change in plasma inorganic phosphorus and an increase in faecal excretion of phosphorus cause reduced incidence of phosphatic calculi in sheep.

Kallfelz *et al.* (1986) reported in calves that calculogenic effect of a ration due to either high magnesium (1.4 per cent) alone or in combination with high phosphorus (1.6 per cent), was somewhat overcome by high level of dietary calcium (1.8 per cent).

2.4.2 Ammonium chloride

Crookshank *et al.* (1960) reported that supplementation of ammonium chloride at the rate of 90 g per head per day and phosphoric acid at the rate of 80 g per head per day decreases

both calculi formation and pH of urine in steers fed with fattening ration composed of cottonseed meal, ground milo, sumac silage and alfalfa hay.

Bushman *et al.* (1967,1968) reported that supplementation of ammonium chloride at the rate of 1-1.5 per cent to high phosphorus diet known to produce calculi in lambs significantly reduced the incidence of urinary calculi, possibly by reducing the urine pH.

Crookshank (1970) studied the anticalculogenic effect of different ammonium salts such as ammonium chloride, ammonium sulfate, diammonium phosphate and ammonium polyphosphate by supplementing it to known calculogenic ration and suggested that ammonium chloride as a primary compound to prevent calculi with an advantage of increased feed efficiency and weight gain in lambs.

In vitro study on the mechanism of ammonium chloride on the prevention of urolithiasis in fattening cattle by Yano and Kawashima (1977) found that among the effect of ammonium chloride, lowering the urine pH would play the most important part in the prevention of urolithiasis.

Stewart *et al.* (1991) found that addition of ammonium chloride at one per cent level in the ration of lambs tend to

lower the incidence of silica urolithiasis and suggested the acid forming potential of the diet.

2.4.3 Sodium chloride (salt)

Elam *et al.* (1958) observed that supplementation of salt at 10 per cent level to sheep ration reduced the incidence of urolithiasis by increased output of urine, which significantly lowered the pH of urine to 7.3 compared with 7.9 in unsupplemented group.

Udall (1959) reported that supplementation of 10 per cent salt to a calculogenic ration fed to wethers reduced the incidence of urolithiasis by increasing water turnover than the group fed without added salt.

Udall (1962) from a factorial trial in wether lambs when fed with 1, 4 and 7 per cent of sodium chloride and the ash of urine when made acidic by hydrogen phosphate or alkaline by giving potassium bicarbonate on slaughter, calculi recovered from kidney of lambs fed with 4 per cent sodium chloride or more, on diet produced either acidic or alkaline ash. The pH of urine was found to differ significantly between those diets obtained by the effect of salts suggested that emphasis on pH of urine alone is not warranted, but the activity of all the ions present must be considered.

Bailey *et al.* (1963) reported that for yearling steer (300 kg) dietary consumption of 50 g of salt daily had no effect on the formation of silicious calculi whereas at 200 g daily intake, the occurrence of calculi was significantly reduced and at 300 g daily, calculus formation was almost eliminated.

Udall *et al.* (1965) and Udall and Chow (1965) reported that supplementation of sodium chloride, sodium carbonate or potassium carbonate to wether lambs implanted with stilbesterol caused reduction in the incidence of calculi in those animals which excrete more chloride ion in urine and suggested that chloride ion in the salt prevent the formation of calculi. He also suggested that salt displace magnesium and phosphorus from the centre of nucleation on the matrix and prevent crystallization and calculi development. Lack of stone formation could be explained on diuresis caused by salt and consequent dilution of urine.

Bailey (1976) suggested that for calves on native range, the provision of supplementation containing 12-20 per cent salt was effective in eliminating silicious calculi.

Koutinas *et al.* (1989) recorded an outbreak of phosphatic urolithiasis in fattening male lambs and he suggested the preventive measures including increased percentage of dietary

salt and adding ammonium chloride at the rate of 2 per cent of the ration.

Singh and Hussain (1992) observed that high sodium intake increased calcium and sulfur excretion in urine and the risk of high calcium excretion was counteracted by high sulfur excretion thereby eliminated the risk of stone formation.

Apart from the above chemical agents, some other substances can also be suggested for its anticalculogenic activity.

Van Reen *et al.* (1965) reported that rats fed basal diet with 30 per cent casein developed urinary calculi the incidence was significantly reduced by adding either 0.51 per cent methionine or 1.48 per cent lysine hydrochloride and the calculi were prevented when both were given.

Emerick *et al.* (1972) observed in sheep that potassium chloride at 1 or 2 per cent level in the ration provided some protection against the calculogenic effect of 0.5 per cent phosphorus but had no effect when phosphorus level was 0.7 per cent in the ration.

James (1973) reported that the calculogenic effect of high magnesium diet for goats can be prevented by supplementation of sulfur (1.044 per cent) in the ration,

render the urine acidic, prevented crystalluria and manifestation of any clinical signs of obstructive urolithiasis. He also found that supplementation of Vitamin D to a calculogenic ration with high magnesium have some protective action.

Fluss (1981) reported that sodium acid phosphate at the rate of 6 g per head orally twice daily was successful for oxalate urolithiasis in goats.

Blood and Radostits (1989) mentioned that although the importance of vitamin A in the production of this urolithiasis has been decreased in recent years, an adequate intake should be ensured especially during drought period and when animals fed on high grain ration in feedlots.

2.4.5 Plant agents

With regard to plant agents very limited work has been carried out towards amelioration of urinary calculi in animals.

In vitro effect of tamarind (*Tamarindus indicus* L.) on inhibitory activity in urine has been investigated by Singh *et al.* (1987) and observed that tamarind intake has got influence on calcium oxalate crystallization in human beings.

Beneficial result have been obtained with extract of banana stem juice (Family - Musaceae) on experimentally induced hyperoxaluric rats. According to Poonguzhali and Varalakshmi (1992) the extract had very good effect on lowering the oxalate forming enzyme activity and inorganic constituents of hyperoxaluric rats.

Das (1956) reported successful treatment of urethral calculi in a bullock with a decoction of horse gram (*Dolichos biflorus*) prepared by boiling one pound of horse gram in four pounds of water and administered orally after boiled gram was strained.

An Ayurvedic medicine `Cystone', prescribed to kidney stones comprising 15 plant extracts including *Dolichos biflorus* and mineral products was found to contain water soluble compound which inhibit initial precipitation of calcium and phosphorus ions in the form of mineral phase, bound to the organic matrix and subsequent growth of preformed mineral phase. The aqueous extract could stimulate demineralization of the matrix bound mineral phase (Jethi *et al.*, 1983).

Joshi *et al.* (1988a, b) studied the effect of Cystone on urine crystalization in bullocks and reported that it controls the crystaluria in animals after a treatment period of 15 days. The results tend to show that Cystone decreases

supersaturation by decreased excretion of urine calcium and pH of urine, probably as a result of its diuretic activity and may be of use in prevention of urolithiasis in bullocks.

In vitro effect of *D. biflorus* seed on crystalization of calcium phosphate, a major constituents of kidney stones has been studied by Archana and Singla (1994) who concluded that inhibitors of crystallization in the extract of *D. biflorus* were water soluble, labile, heat stable, polar, non-tannin and likely to be non-protein in nature, showing beneficial effect on dissolution of urinary calculi by inhibiting precipitation of calcium and phosphate ions.

Eventhough there are many reports on the anticalculogenic and calculolytic properties of various chemical or plant agents on different species of animals, reports on similar work in caprines are rather scanty in the literature. Hence, it is necessary to take up studies on the effect of incorporation of ammonium chloride and horse gram extract in the ration on the amelioration of urolithiasis in goats maintained on a calculogenic ration having high magnesium.

Materials and Methods

3. MATERIALS AND METHODS

3.1 Experimental animals

Eighteen male Malabari goats, 9-12 months of age weighing on an average of 19.4 kg, after being dewormed and sprayed against ectoparasites, were used in the present investigation. The animals selected and procured from the herd of goats maintained by the University Goat and Sheep Farm, Mannuthy, formed the experimental subjects for the study. They were distributed randomly to three experimental groups of six animals each (groups I, II and III) as uniformly as possible, with regard to age and body weight. All the animals were maintained on identical conditions of management and were fed individually (Plate 1). Wholesome water was made available at all times. The experimental duration lasted for 84 days excluding initial and final metabolism trials.

3.2 Experimental ration

The concentrate mixture was prepared by using the following conventional feed ingredients:

Wheat	-	30 per cent
Bengal gram	-	30 "
Gingelly oil cake	-	30 "

Plate 1. Individual feeding of experimental goats

Black gram bran	-	8	per cent
Salt	-	0.5	"
Mineral mixture*	-	1.5	"

* Mineral mixture was guaranteed to contain: calcium 24%, phosphorus 12%, manganese 0.150%, copper 0.150%, zinc 0.380%, magnesium 6.500%, iron 0.500%, iodine 0.030%, cobalt 0.020%, sulfur 0.500%, acid insoluble ash 2.000% max and fluorine 0.040% max.

Throughout the experimental period, a roughage to concentrate ratio of 1:4 was maintained.

Hybrid Napier grass was chopped fed to the animals to meet roughage portion of the ration.

The percentage chemical composition (on dry matter basis) of the compounded feed and grass, on proximate analysis are shown below:

Chemical components	Concentrate	Roughage (grass)
Dry matter	93.17	34.93
Crude protein	22.94	9.75
Crude fibre	10.01	43.69
Ether extract	4.12	3.07
Nitrogen free extract	51.66	39.64
Total ash	11.27	3.85
Calcium	0.84	0.14
Phosphorus	0.51	0.18
Magnesium	0.31	0.23

The calcium and phosphorus level has been raised to an estimated level of 1.194 per cent and 0.578 per cent by adding calcium oxide and phosphorus pentoxide to the above ration.

With this compounded feed, three rations were prepared as follows: The basal concentrate ration known to be calculogenic was computed by using the concentrate mixture added with magnesium oxide to raise the magnesium to the estimated level of 1.202 per cent. The experimental ration B was prepared by using the basal concentrate ration A fortified with ammonium chloride to the extent of one per cent. Experimental ration C was formed by feeding the basal concentrate ration A along with horse gram extract (*Dolichos biflorus*) to the extent of one litre per animal per day in place of drinking water.

Everyday, the horse gram (*Dolichos biflorus*) extract (decoction) was prepared by boiling 1.5 kg horse gram with six litre of drinking water (horse gram and water in the ratio of 1:4) for 60 minutes, then allowed to cool overnight. Next day morning, the extract was strained and given to animals in ration C at the rate of one litre per animal per day.

Three experimental groups (I, II and III) of six animals each were fed with the rations A, B and C respectively. Individual animal feed allowance for maintenance was calculated based on the body weight.

3.3 Methods

The goats were weighed at weekly intervals. Daily feed and grass intake and balance of both were recorded throughout the course of study. Digestion cum balance trials were carried out initially after 20 days of acclimatization period before feeding the experimental rations and afterwards at monthly intervals till the termination of feeding trial. During this period quantitative and separate collection of urine and faeces were carried out with a collection period extended for five consecutive days, using metabolism cages specially fabricated for the purpose (Plate 2).

During the progress of experiment, animals were closely observed for any signs of urinary tract obstruction.

3.3.1 Collection and sampling of faeces and urine

All precautions were taken to ensure the collection of faeces and urine quantitatively. The faeces was collected manually as and when it was voided. Urine collection device has been rinsed frequently with distilled water. Concentrated sulphuric acid (25 per cent) at the rate of 20 ml was added to each bottle before the collection of urine. At 9.30 a.m. everyday, the faeces and urine voided during the previous 24 hours were measured accurately. Representative samples of both faeces and urine were taken separately at the rate of

Plate 2. **Experimental goats on metabolism trial**

10 per cent of the total quantity voided and were stored in deep freezer. The samples collected from each animal were preserved during the entire collection period and later pooled and used for further analysis.

The feed sample collected during metabolism trial were subjected to proximate analysis as per standard procedure (AOAC, 1990).

Protein in feed, faeces and urine were analysed using kjeltec-2000 digestion and distillation unit.

Calcium and magnesium content of feed, faeces and urine samples were estimated by using Atomic Absorption Spectrophotometer (Parkin Elmer, Model 3110). Urine phosphorus was determined colorimetrically with Spectronic-20 (Milton Roy Co., USA) using commercial phosphorus kit (Qualigens Diagnostics).

Feed and faecal phosphorus were estimated colorimetrically by nitric acid vanado-molibdate method (AOAC, 1990).

Fresh urine samples were collected at monthly intervals to determine the pH and examined microscopically for the presence of crystals. The crystals were collected and

subjected to qualitative chemical analysis (Spot tests by Winer, 1959).

3.3.2 Haematological studies

Blood samples were collected at monthly intervals by jugular puncture into sterile citrated tubes and in clean dry tubes for serum separation.

Total erythrocyte count was made by using improved Neubauer counting chamber with 1:200 dilution of blood using Hayem's fluid as diluent.

Total leucocyte cell counts were made by using Truck's fluid as the diluent with 1:20 dilution.

Haemoglobin content was determined by Acid haematin method using haemometer with permanent coloured glass comparison standard.

Plasma protein values were determined colorimetrically by Biuret method using total protein kit supplied by Stangen Immunodiagnostics.

Serum calcium and magnesium were estimated by Atomic Absorption Spectrophotometer (Parkin Elmer, Model 3110). Serum inorganic phosphorus was estimated colorimetrically

(modified metol method) by using commercial phosphorus kit (Qualigen Diagnostics).

3.3.3 Slaughter study

At the termination of experiment, all the animals were slaughtered and the urinary organs were collected and subjected to detailed examination for the presence of gross calculi and other gross lesions. The calculi materials were collected and subjected to spot tests (Winer, 1959) for identification of its chemical composition.

3.3.4 Histopathological study

The specimens from urinary organs viz., kidney, ureter and bladder were collected and preserved in neutral buffered formalin (10 per cent) for histopathological study. These tissues were processed with routine paraffin embedding method and sections were stained with haematoxylin and eosin.

The data gathered during the course of present investigation have been analysed statistically as per the method described by Snedecor and Cochran (1980).

Results

4. RESULTS

4.1 Body weight gain

The data on average weekly body weight gain of animals maintained on three dietary regimes recorded during the entire period of study are presented in Table 1 and are represented by Figure 1. The summarised data on average initial body weight, final body weight, average cumulative weight gain and average daily gain of animals are presented in Table 2 and statistical analysis of the data are set out in Tables 3 and 4.

4.2 Dry matter intake, feed efficiency and protein efficiency

Average daily dry matter intake, feed efficiency values and protein efficiency values recorded during the experimental period of 12 weeks of study are presented in Tables 5, 6 and 7 respectively, summarised in Table 8 and statistical analysis presented in Tables 9, 10 and 11. Data on feed efficiency and protein efficiency are represented as Figures 2 and 3.

4.3 Haematological observations

The data on haematological parameters such as TEC, TLC, haemoglobin and total protein are set out in Tables 12 to 15,

summarised in Table 16 and their statistical analysis were given in Table 17. Data on serum calcium, inorganic phosphorus, and magnesium are presented in Tables 18, 19 and 20 respectively, represented in Figures 4, 5 and 6 respectively, summarised in Table 21, and their statistical analysis are set out in Table 22.

4.4. Balance studies

Results obtained by the balance studies conducted at monthly intervals with regard to calcium, phosphorus, magnesium and nitrogen are set out in Tables 23 to 26, 27 to 30, 31 to 34 and 35 to 38 respectively. Consolidated data (g/day) on urine and faecal excretion of calcium, phosphorus, magnesium and nitrogen are presented in Tables 39 and 40 and their statistical analysis set out in Tables 42 and 43. Urine concentration (mg/dl) of calcium, phosphorus and magnesium are represented as Figures 7, 8 and 9 respectively. Data on per cent retention of calcium, phosphorus, magnesium and nitrogen are represented by Figures 10 to 13, summarised in Table 41 and their statistical analysis are set out in Table 44.

Table 1. Average weekly body weight (kilogram) of animals maintained on the experimental rations (Rations A, B and C)

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
0 day	22.39±0.93	22.53±1.24	22.30±1.01
1 week	22.85±1.04	22.99±1.30	22.75±1.04
2	23.29±1.25	23.44±1.35	23.20±1.08
3	23.73±1.29	23.88±1.35	23.64±1.13
4	24.16±1.34	24.32±1.39	24.07±1.17
5	24.58±1.39	24.75±1.43	24.50±1.22
6	24.99±1.42	25.18±1.49	24.93±1.24
7	25.41±1.45	25.61±1.55	25.35±1.28
8	25.84±1.46	26.05±1.59	25.78±1.31
9	26.28±1.45	26.50±1.64	26.20±1.37
10	26.72±1.48	26.95±1.69	26.63±1.41
11	27.15±1.46	27.39±1.73	27.06±1.46
12	27.59±1.52	27.82±1.79	27.47±1.51

FIG. 1 AVERAGE WEEKLY BODY WEIGHT (Kg) OF GOATS MAINTAINED ON THREE RATIONS

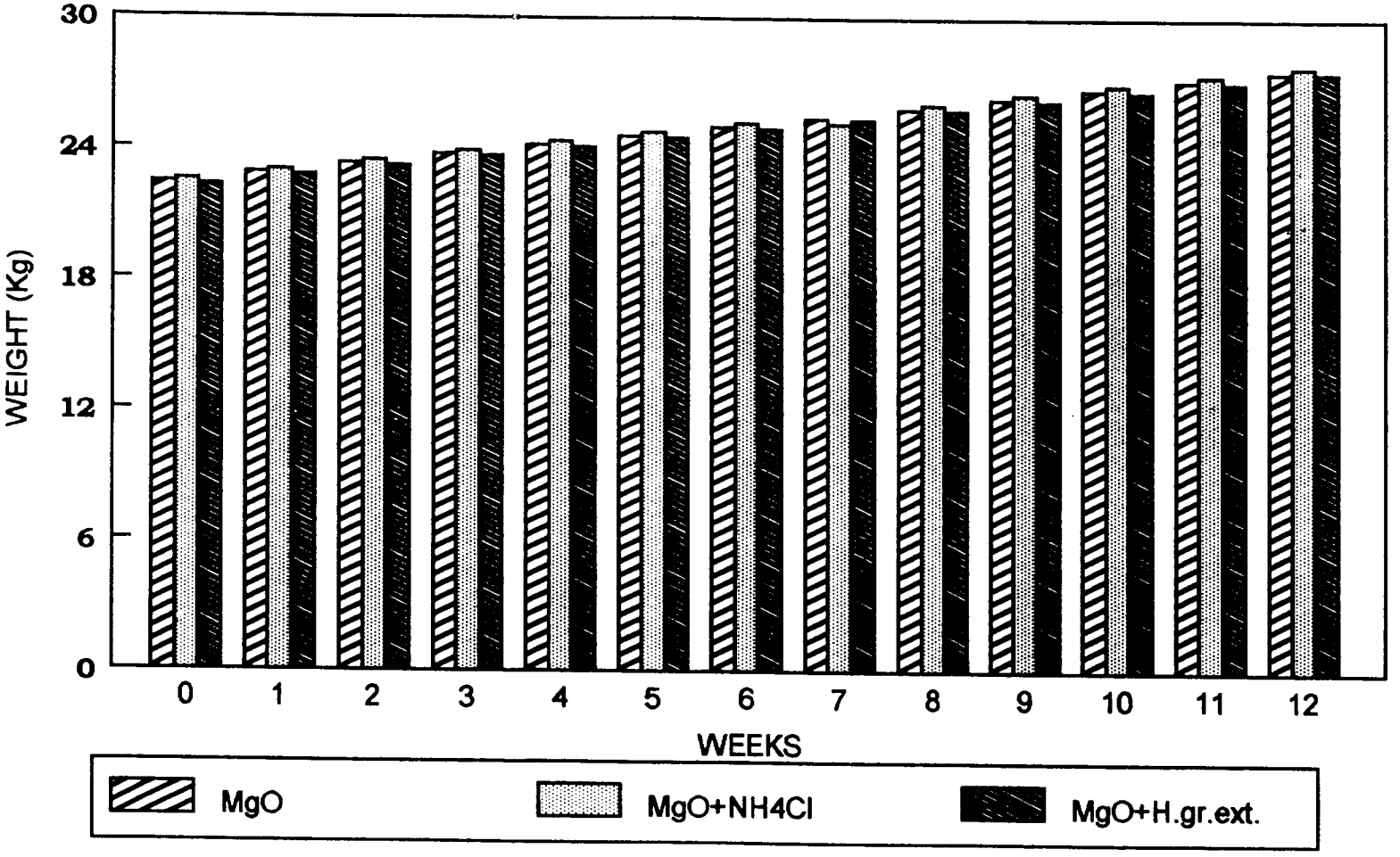


Table 2. Summarised data on average initial body weight, average final body weight, average cumulative weight gain and average daily gain of animals maintained on three dietary regimes (Rations A, B and C)

Treatments	Ration A containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202%	Ration B containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Ammonium chloride-1%	Ration C containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Horse gram extract 1 lit/animal/day
Number of animals	6	6	6
Initial body weight (kg)	22.39±0.93	22.53±1.24	22.30±1.01
Final body weight (kg)	27.59±4.52	27.82±1.79	27.47±1.51
Cumulative weight gain 84 days (kg)	5.20±0.429	5.29±0.445	5.17±0.387
Average daily gain (g)	61.90±0.004	62.98±0.006	61.55±0.002

Table 3. Analysis of variance - Cumulative weight gain

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Between	2	0.00075	0.0003	3.00NS
Within	33	0.004	0.0001	
Total	35	0.00475		

Table 4. Analysis of variance - Average daily gain

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Between	2	13.26	6.63	2.41NS
Within	33	90.68	2.75	
Total	35	103.94		

NS - Not significant

Table 5. Average daily dry matter intake (g) of the animals maintained on the experimental rations (Rations A, B and C)

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
0-7 days	727.64	738.06	720.42
7-14	755.40	479.08	740.78
14-21	781.95	787.82	766.18
21-28	808.92	797.68	790.54
28-35	855.65	856.87	835.87
35-42	916.82	890.26	884.75
42-49	953.56	939.27	920.13
49-56	1017.31	998.19	990.56
56-63	1056.37	1033.38	998.12
63-70	1066.53	1052.60	1047.04
70-77	1075.40	1070.59	1078.12
77-84	1127.32	1102.03	1054.00

Table 6. Average weekly feed efficiency of animals maintained on three dietary regimes (unit dry matter intake per unit gain)

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
0-7 days	11.07	11.23	11.20
7-14	12.02	11.65	11.52
14-21	12.44	12.53	12.19
21-28	13.17	12.69	12.86
28-35	14.26	13.86	13.60
35-42	15.65	14.49	14.40
42-49	15.89	15.29	15.33
49-56	16.56	15.88	15.96
56-63	16.80	16.07	16.63
63-70	16.96	16.37	17.04
70-77	17.50	17.03	17.55
77-84	17.93	17.94	17.99

FIG. 2 AVERAGE WEEKLY FEED EFFICIENCY OF GOATS MAINTAINED ON THREE RATIONS

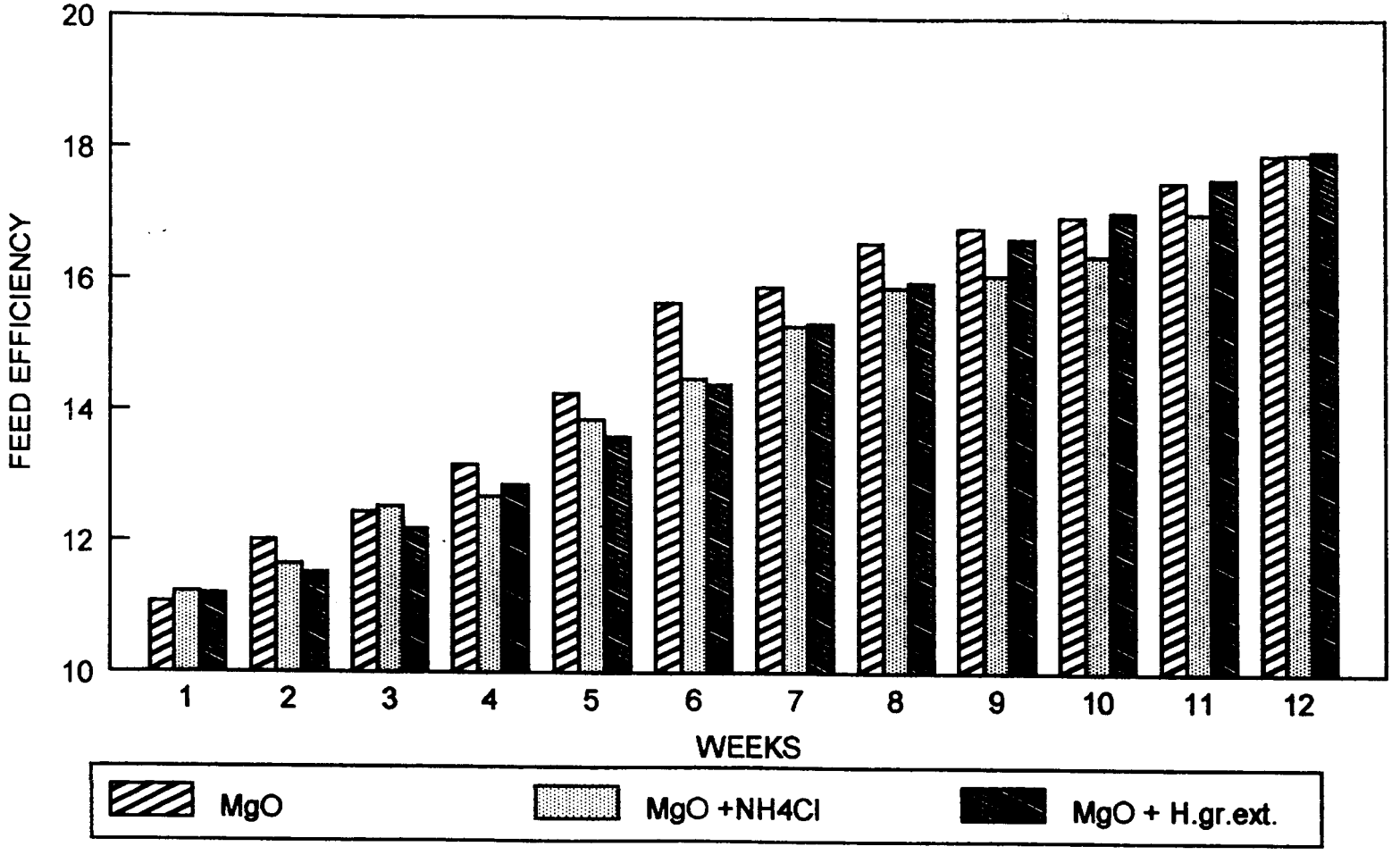


Table 7. Average weekly protein efficiency of animals maintained on three dietary regimes (unit protein consumed per unit gain)

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
0-7 days	1.97	1.95	1.99
7-14	2.14	2.03	2.05
14-21	2.21	2.18	2.17
21-28	2.35	2.21	2.29
28-35	2.54	2.43	2.43
35-42	2.79	2.53	2.57
42-49	2.83	2.66	2.73
49-56	2.95	2.76	2.84
56-63	2.99	2.80	2.96
63-70	3.02	2.85	3.04
70-77	3.12	2.96	3.13
77-84	3.19	3.12	3.20

FIG. 3 AVERAGE WEEKLY PROTEIN EFFICIENCY OF GOATS MAINTAINED ON THREE RATIONS

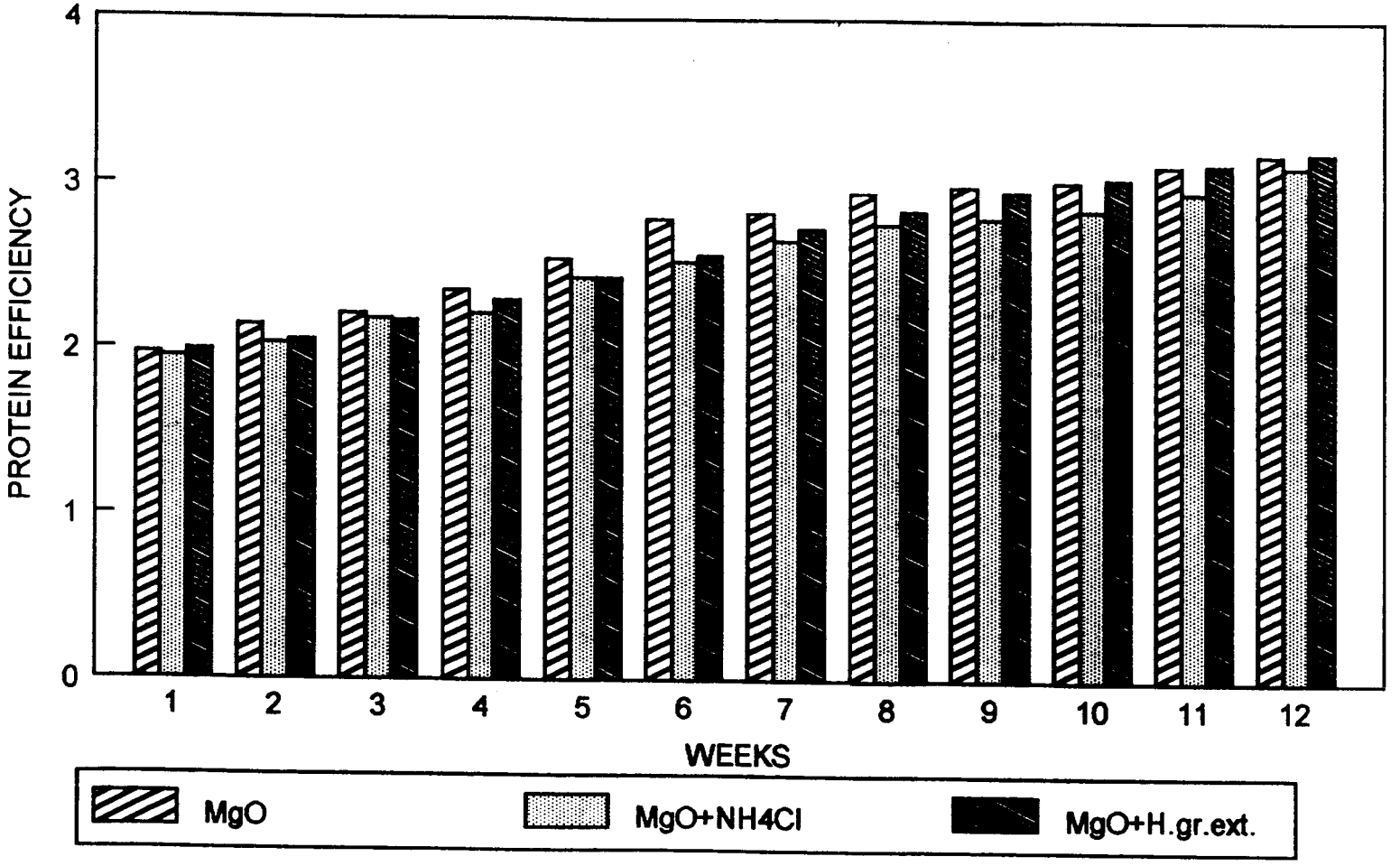


Table 8. Summarised data on daily dry matter consumption, dry matter consumption per 100 kg body weight, average cumulative feed efficiency and average cumulative protein efficiency of goats maintained on three dietary regimes (Rations A, B and C)

Treatments	Ration A containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202%	Ration B containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Ammonium chloride-1%	Ration C containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Horse gram extract 1 lit/animal/day
Number of animals	6	6	6
Average daily dry matter consumption (g)	928.57	917.98	901.38
Dry matter consumption per 100 kg body weight	3.71	3.64	3.62
Average cumulative feed efficiency	15.02±0.141	14.58±0.109	14.69±0.150
Average cumulative protein efficiency	2.69±0.022	2.60±0.20	2.62±0.023

Table 9. Analysis of variance - Average daily dry matter consumption

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Between	2	4510.51	2255.26	0.125NS
Within	33	594259.07	18007.85	
Total	35	598769.58		

Table 10. Analysis of variance - Average feed efficiency

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Between	2	1.24	0.63	0.117NS
Within	33	176.85	5.36	
Total	35	178.09		

Table 11. Analysis of variance - Average protein efficiency

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
Between	2	0.11	0.06	0.353NS
Within	33	5.54	0.17	
Total	35	5.65		

NS -Not significant

Table 12. Average total erythrocyte count (TEC) ($10^6/mm^3$) of animals maintained on the experimental rations (Rations A, B and C)

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
0 day	12.41±0.72	12.45±0.63	12.92±0.46
28 day	13.25±0.54	12.25±0.37	13.97±0.35
56 day	12.58±0.68	13.00±0.55	12.04±0.59
84 day	12.70±0.63	13.34±0.66	12.84±0.64

Table 13. Average total leucocyte count (TLC) ($10^3/mm^3$) of animals maintained on the experimental rations (Rations A, B and C)

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
0 day	11.93±0.51	12.16±0.72	11.76±0.44
28 day	10.54±0.85	11.40±0.24	11.10±0.42
56 day	11.94±0.30	12.70±0.38	11.45±0.36
84 day	11.08±0.69	11.86±0.59	11.48±0.71

Table 14. Average haemoglobin concentration (g/dl) of animals maintained on the experimental rations (Rations A, B and C)

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
0 day	8.37±0.26	8.57±0.27	8.53±0.22
28 day	8.41±0.35	8.04±0.28	8.26±0.38
56 day	8.63±0.75	8.25±0.16	8.44±0.46
84 day	8.27±0.22	8.40±0.23	8.23±0.17

Table 15. Average plasma protein concentration (g/dl) of animals maintained on the experimental rations (Rations A, B and C)

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
0 day	7.77±0.29	7.38±0.62	7.02±0.17
28 day	7.92±0.18	7.83±0.21	7.45±0.26
56 day	7.49±0.26	7.26±0.63	7.57±0.43
84 day	8.09±0.36	7.43±0.48	7.79±0.46

Table 16. Summarised data on TEC, TLC, haemoglobin and plasma protein values of goats maintained on three experimental rations for a period of 84 days

Treatments	Ration A containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202%	Ration B containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Ammonium chloride-1%	Ration C containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Horse gram extract 1 lit/animal/day
Number of animals	6	6	6
	TEC ($x10^6/mm^3$)		
Initial (0 day)	12.41±0.72	12.45±0.63	12.92±0.46
Final (84 day)	12.70±0.63	13.34±0.66	12.84±0.64
Difference	0.29	0.89	0.08
	TLC ($x10^3/mm^3$)		
Initial (0 day)	11.93±0.51	12.16±0.72	11.76±0.44
Final (84 day)	11.08±0.69	11.86±0.59	11.48±0.71
Difference	0.85	0.30	0.28
	Haemoglobin (g/dl)		
Initial (0 day)	8.37±0.26	8.57±0.27	8.53±0.22
Final (84 day)	8.27±0.22	8.40±0.23	8.23±0.17
Difference	0.10	0.17	0.30
	Plasma protein (g/dl)		
Initial (0 day)	7.77±0.29	7.38±0.62	7.02±0.17
Final (84 day)	8.09±0.36	7.43±0.48	7.97±0.46
Difference	0.32	0.05	0.95

Table 17. Analysis of variance - TEC, TLC, haemoglobin and total protein

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
		TEC		
Between	2	2.162	1.041	0.706 NS
Within	15	22.974	1.532	
Total	17	26.136		
		TLC		
Between	2	1.451	0.725	0.453 NS
Within	15	24.046	1.603	
Total	17	25.497		
		Haemoglobin		
Between	2	0.573	0.287	1.226 NS
Within	15	3.507	0.234	
Total	17	4.080		
		Plasma protein		
Between	2	0.457	0.228	0.230
Within	15	14.895	0.993	
Total	17	15.352		

Table 18. Average serum calcium concentration (mg/dl) of animals maintained on the experimental rations (Rations A, B and C)

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
0 day	10.41±0.18	10.32±0.21	10.50±0.16
28 day	8.93±0.44	9.45±0.17	8.73±0.25
56 day	8.27±0.12	8.79±0.28	8.31±0.36
84 day	7.08±0.23	8.63±0.20	8.32±0.11

Table 19. Average serum inorganic phosphorus concentration (mg/dl) of animals maintained on the experimental rations (Rations A, B and C)

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
0 day	5.35±0.11	5.31±0.16	5.58±0.21
28 day	5.94±0.21	5.62±0.23	5.75±0.34
56 day	6.72±0.34	5.73±0.40	6.30±0.32
84 day	7.67±0.07	6.10±0.10	6.69±0.08

FIG. 4 AVERAGE SERUM CALCIUM CONCENTRATION (mg/dl) OF GOATS FED THREE RATIONS

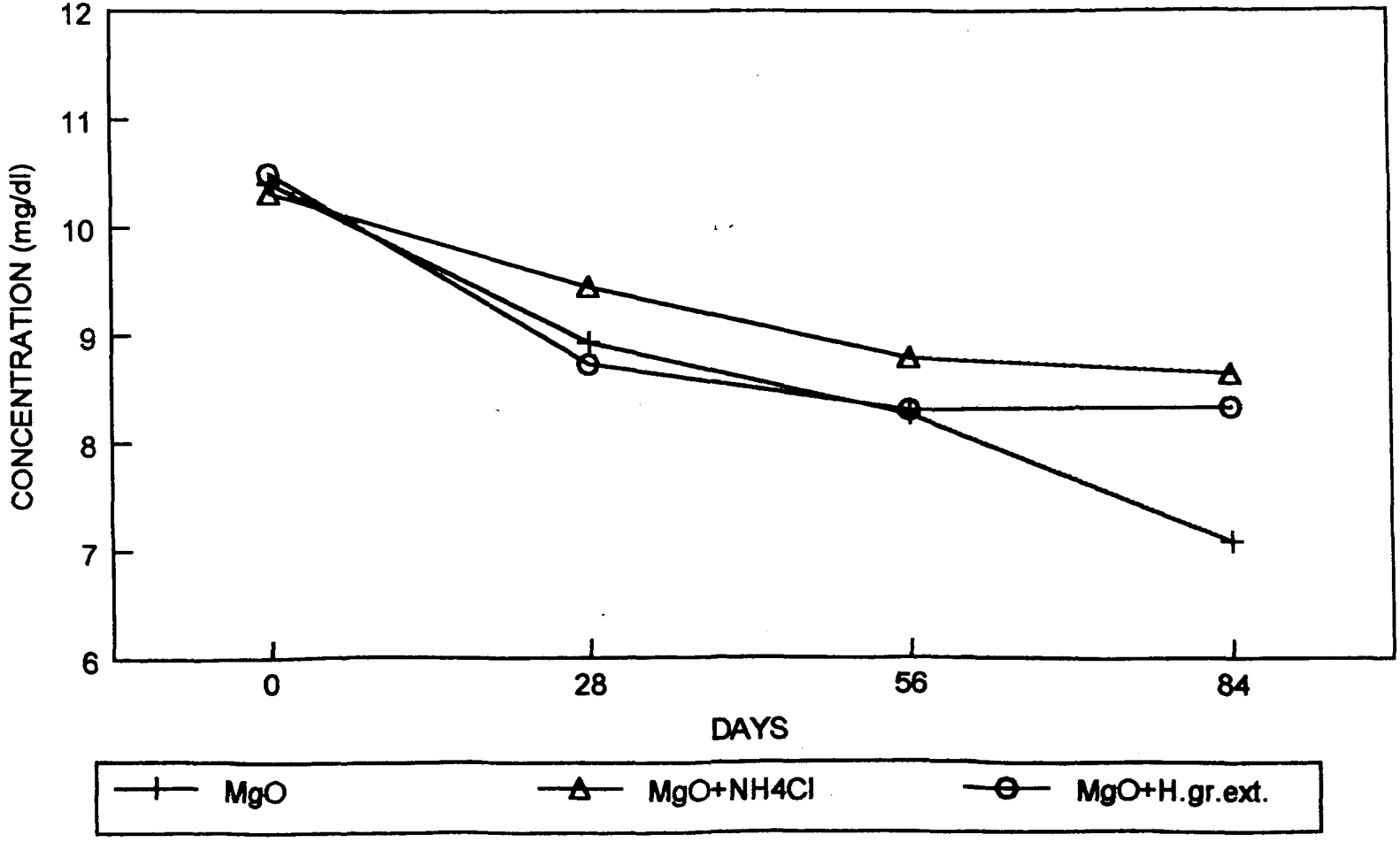


FIG. 5 AVERAGE SERUM PHOSPHORUS CONCENTRATION (mg/dl) OF GOATS FED THREE RATIONS

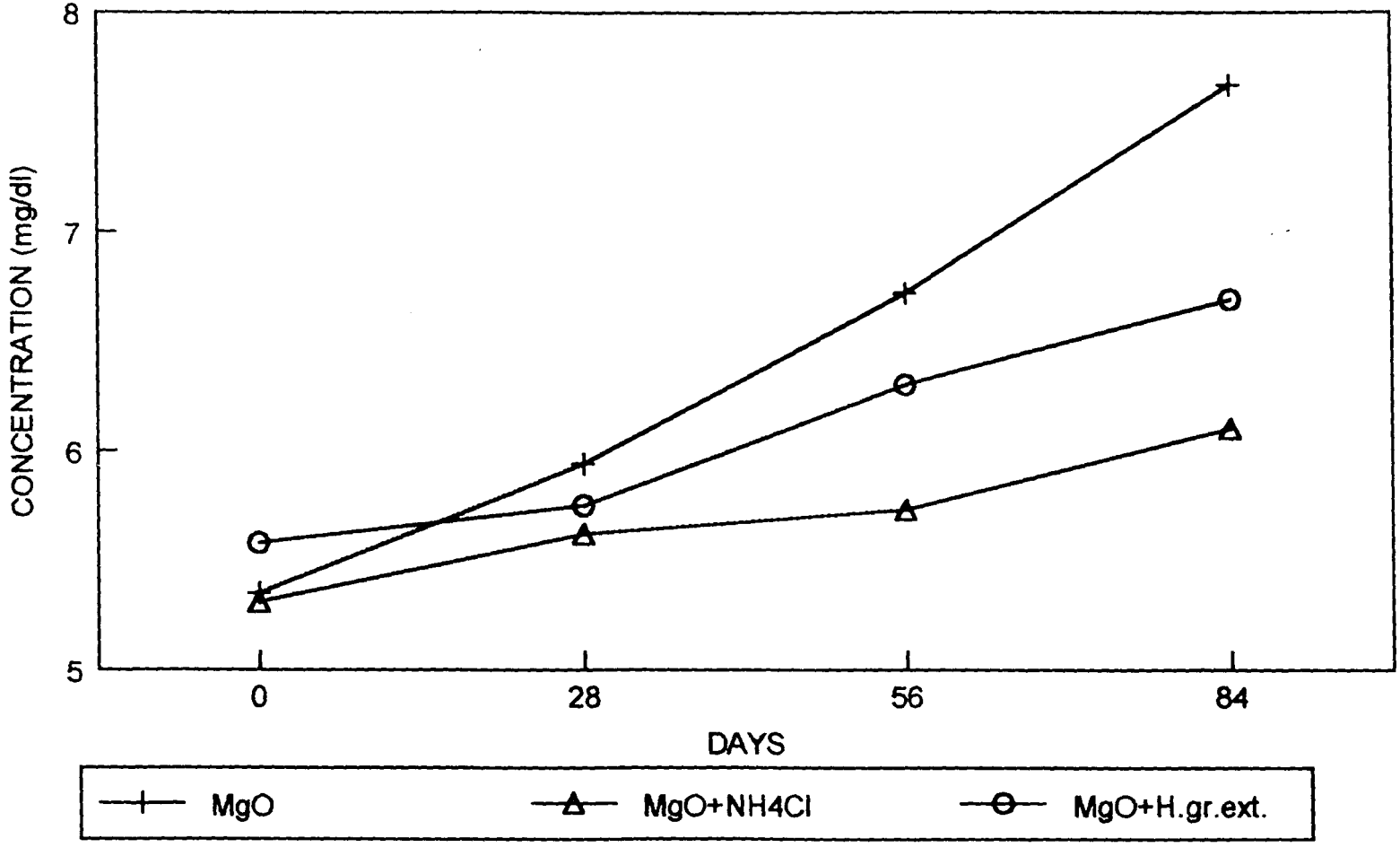


Table 20. Average serum magnesium concentration (mg/dl) of animals maintained on the experimental rations (Rations A, B and C)

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
0 day	2.60±0.12	2.53±0.20	2.67±0.17
28 day	4.78±0.28	3.41±0.26	3.99±0.28
56 day	5.65±0.21	4.16±0.32	4.71±0.36
84 day	6.08±0.15	4.70±0.14	5.50±0.12

FIG. 6 AVERAGE SERUM MAGNESIUM CONCENTRATION (mg/dl) OF GOATS FED THREE RATIONS

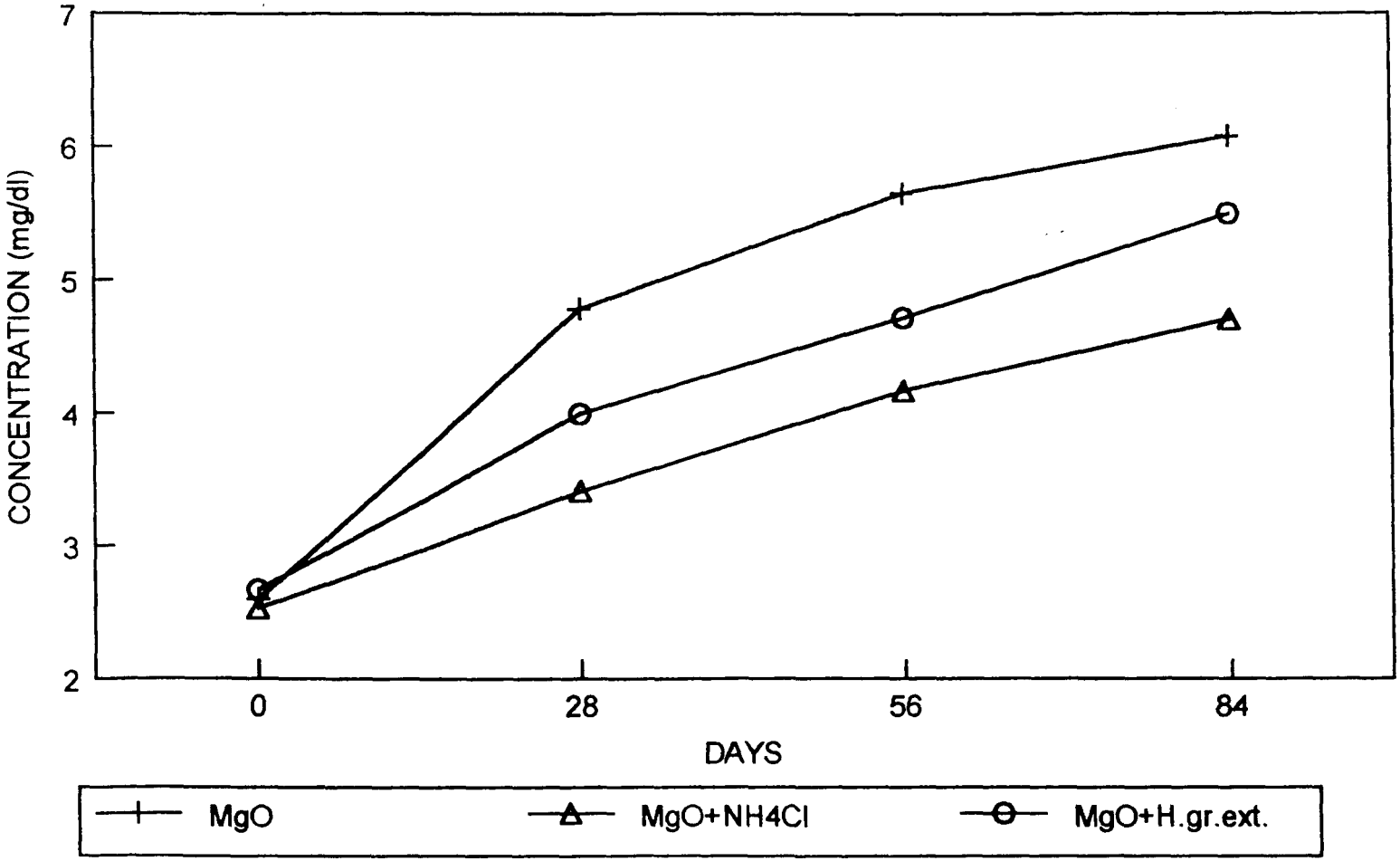


Table 21. Summarised data on serum calcium, serum inorganic phosphorus and serum magnesium values of goats maintained on three experimental rations for a period of 84 days

Treatments	Ration A containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202%	Ration B containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Ammonium chloride-1%	Ration C containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Horse gram extract 1 lit/animal/day
Number of animals	6	6	6
Serum calcium (mg/dl)			
Initial (0 day)	10.41±0.18	10.32±0.21	10.50±0.16
Final (84 day)	7.08±0.23	8.63±0.20	8.32±0.11
Difference	3.33	1.69	2.18
Serum inorganic phosphorus (mg/dl)			
Initial (0 day)	5.35±0.11	5.31±0.16	5.58±0.21
Final (84 day)	7.67±0.07	6.10±0.10	6.69±0.08
Difference	2.32	0.79	1.11
Serum magnesium (mg/dl)			
Initial (0 day)	2.60±0.12	2.53±0.20	2.67±0.17
Final (84 day)	6.08±0.15	4.70±0.14	5.50±0.12
Difference	3.48	2.17	2.83

Table 22. Analysis of variance - serum calcium, serum inorganic phosphorus and serum magnesium

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
		Serum calcium		
Between	2	8.434	4.217	15.907**
Within	15	3.977	0.265	
Total	17	12.411		
		Serum inorganic phosphorus		
Between	2	6.347	3.173	14.660**
Within	15	3.247	0.216	
Total	17	9.594		
		Serum magnesium		
Between	2	3.990	1.995	16.038**
Within	15	1.835	0.122	
Total	17	5.825		

** Significant at 1% level

Table 23. Data on calcium balance and per cent calcium retention of the animals in three groups maintained on conventional concentrate ration during initial (0 day) metabolism trial

Groups	I	II	III
Number of animals	6	6	6
Calcium intake (g/day)	9.091	8.139	8.391
Calcium outgo			
Faecal (g/day)	3.352	2.925	3.147
Urinary (g/day)	0.104	0.109	0.108
(mg/dl)	7.542	7.334	7.956
Total (g/day)	3.456	3.034	3.247
Calcium balance	5.635	5.105	5.144
Per cent retention of calcium	62.05	62.71	61.04

Table 24. Data on calcium balance and per cent calcium retention of the animals in three groups maintained on three dietary regimes during second (28th day) metabolism trial

Treatment	Ration A	Ration B	Ration C
Number of animals	6	6	6
Calcium intake (g/day)	10.028	9.970	9.728
Calcium outgo			
Faecal (g/day)	3.760	3.551	3.594
Urinary (g/day)	0.134	0.119	0.124
(mg/dl)	8.201	6.324	5.701
Total (g/day)	3.894	3.670	3.718
Calcium balance	6.134	6.300	6.010
Per cent retention of calcium	61.17	63.19	61.78

Table 25. Data on calcium balance and per cent calcium retention of the animals in three groups maintained on three dietary regimes during third (56th day) metabolism trial

Treatment	Ration A	Ration B	Ration C
Number of animals	6	6	6
Calcium intake (g/day)	12.436	11.920	11.353
Calcium outgo			
Faecal (g/day)	4.769	4.109	4.118
Urinary (g/day)	0.169	0.126	0.136
(mg/dl)	11.715	6.966	8.136
Total (g/day)	4.938	4.235	4.254
Calcium balance	7.498	7.685	7.099
Per cent retention of calcium	60.29	64.47	62.53

Table 26. Data on calcium balance and per cent calcium retention of the animals in three groups maintained on three dietary regimes during final (84th day) metabolism trial

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
Calcium intake (g/day)	13.245	13.349	12.129
Calcium outgo			
Faecal (g/day)	5.115	4.304	4.472
Urinary (g/day)	0.191	0.128	0.151
(mg/dl)	12.862	5.587	7.968
Total (g/day)	5.305	4.432	4.622
Calcium balance	7.939	8.917	7.756
Per cent retention of calcium	59.85	66.55	62.65

Table 27. Data on phosphorus balance and per cent phosphorus retention of the animals in three groups maintained on conventional concentrate ration during initial (0 day) metabolism trial

Groups	I	II	III
Number of animals	6	6	6
Phosphorus intake (g/day)	2.788	2.589	2.682
Phosphorus outgo			
Faecal (g/day)	1.384	1.349	1.218
Urinary (g/day)	0.182	0.197	0.199
(mg/dl)	13.197	13.255	14.661
Total (g/day)	1.566	1.547	1.418
Phosphorus balance (g/day)	1.222	1.163	1.264
Per cent retention of phosphorus	46.64	45.28	47.48

Table 28. Data on phosphorus balance and per cent phosphorus retention of the animals in three groups maintained on three dietary regimes during second (28th day) metabolism trial

Treatment	Ration A	Ration B	Ration C
Number of animals	6	6	6
Phosphorus intake (g/day)	4.954	4.985	4.775
Phosphorus outgo			
Faecal (g/day)	2.572	2.538	2.461
Urinary (g/day)	0.290	0.286	0.231
(mg/dl)	18.237	12.011	10.621
Total (g/day)	2.870	2.764	2.692
Phosphorus balance	2.084	2.221	2.083
Per cent retention of phosphorus	42.07	44.56	43.63

Table 29. Data on phosphorus balance and per cent phosphorus retention of the animals in three groups maintained on three dietary regimes during third (56th day) metabolism trial

Treatment	Ration A	Ration B	Ration C
Number of animals	6	6	6
Phosphorus intake (g/day)	5.990	5.742	5.594
Phosphorus outgo			
Faecal (g/day)	3.502	3.460	2.996
Urinary (g/day)	0.438	0.239	0.258
(mg/dl)	30.362	13.214	15.434
Total (g/day)	3.925	3.221	3.254
Phosphorus balance	2.065	2.521	2.340
Per cent retention of phosphorus	34.47	43.90	41.83

Table 30. Data on phosphorus balance and per cent phosphorus of the animals in three groups maintained on three dietary regimes during final (84th day) metabolism trial

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
Phosphorus intake (g/day)	6.593	6.626	6.243
Phosphorus outgo			
Faecal (g/day)	3.740	3.530	3.613
Urinary (g/day)	0.530	0.231	0.275
(mg/dl)	35.690	10.083	14.512
Total (g/day)	4.270	3.761	3.888
Phosphorus balance (g/day)	2.323	2.865	2.355
Per cent retention of phosphorus	35.24	43.24	37.72

Table 31. Data on magnesium balance and per cent magnesium of the animals in three groups maintained on conventional concentrate ration during initial (0 day) metabolism trial

Groups	I	II	III
Number of animals	6	6	6
Magnesium intake (g/day)	1.844	1.721	1.906
Magnesium outgo			
Faecal (g/day)	1.003	0.977	1.033
Urinary (g/day)	0.220	0.190	0.212
(mg/dl)	15.958	12.785	15.619
Total (g/day)	1.224	1.166	1.246
Magnesium balance (g/day)	0.620	0.554	0.659
Per cent retention of magnesium	33.20	31.86	34.00

Table 32. Data on magnesium balance and per cent magnesium retention of the animals in three groups maintained on three dietary regimes during second (28th day) metabolism trial

Treatment	Ration A	Ration B	Ration C
Number of animals	6	6	6
Magnesium intake (g/day)	10.028	9.987	9.563
Magnesium outgo			
Faecal (g/day)	5.621	5.782	5.221
Urinary (g/day)	0.785	0.653	0.761
(mg/dl)	48.042	34.703	34.988
Total (g/day)	6.406	6.435	5.982
Magnesium balance	3.622	3.552	3.581
Per cent retention of magnesium	36.12	35.57	37.45

Table 33. Data on magnesium balance and per cent magnesium retention of the animals in three groups maintained on three dietary regimes during third (56th day) metabolism trial

Treatment	Ration A	Ration B	Ration C
Number of animals	6	6	6
Magnesium intake (g/day)	12.316	11.921	11.114
Magnesium outgo			
Faecal (g/day)	5.961	6.235	5.531
Urinary (g/day)	1.346	1.121	1.179
(mg/dl)	93.304	61.971	70.528
Total (g/day)	7.307	7.356	6.710
Magnesium balance	5.009	4.565	4.404
Per cent retention of magnesium	40.67	38.29	39.63

Table 34. Data on magnesium balance and per cent magnesium retention of the animals in three groups maintained on three dietary regimes during final (84th day) metabolism trial

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
Magnesium intake (g/day)	13.512	13.565	13.022
Magnesium outgo			
Faecal (g/day)	6.101	6.503	5.973
Urinary (g/day)	1.617	1.580	1.590
(mg/dl)	108.889	68.966	83.905
Total (g/day)	7.718	8.084	7.563
Magnesium balance (g/day)	5.793	5.432	5.459
Per cent retention of magnesium	42.49	40.01	41.80

Table 35. Data on nitrogen balance and per cent nitrogen retention of the animals in three groups maintained on conventional concentrate ration during initial (0 day) metabolism trial

Groups	I	II	III
Number of animals	6	6	6
Nitrogen intake (g/day)	20.863	19.154	19.838
Nitrogen outgo			
Faecal (g/day)	6.904	6.319	6.854
Urinary (g/day)	6.865	6.160	6.618
Total (g/day)	13.997	12.479	13.378
Nitrogen balance	6.865	6.675	6.461
Per cent retention of nitrogen	32.60	34.35	31.43

Table 36. Data on nitrogen balance and per cent nitrogen retention of the animals in three groups maintained on three dietary regimes during second (28th day) metabolism trial

Treatment	Ration A	Ration B	Ration C
Number of animals	6	6	6
Nitrogen intake (g/day)	28.379	27.375	26.146
Nitrogen outgo			
Faecal (g/day)	9.576	8.226	8.493
Urinary (g/day)	8.392	7.675	8.415
Total (g/day)	17.968	15.901	16.908
Nitrogen balance	10.415	10.531	8.942
Per cent retention of nitrogen	36.70	38.47	34.20

Table 37. Data on nitrogen balance and per cent nitrogen retention of the animals in three groups maintained on three dietary regimes during third (56th day) metabolism trial

Treatment	Ration A	Ration B	Ration C
Number of animals	6	6	6
Nitrogen intake (g/day)	32.497	35.898	32.874
Nitrogen outgo			
Faecal (g/day)	10.056	10.667	9.862
Urinary (g/day)	10.142	9.475	9.773
Total (g/day)	20.198	20.142	19.635
Nitrogen balance	12.502	14.782	12.430
Per cent retention of nitrogen	38.48	41.18	37.85

Table 38. Data on nitrogen balance and per cent nitrogen retention of the animals in three groups maintained on three dietary regimes during final (84th day) metabolism trial

Treatments	Ration A	Ration B	Ration C
Number of animals	6	6	6
Nitrogen intake (g/day)	39.544	41.064	37.457
Nitrogen outgo			
Faecal (g/day)	11.610	11.997	11.665
Urinary (g/day)	11.445	10.953	10.907
Total (g/day)	23.060	22.949	22.562
Nitrogen balance	16.485	18.115	14.895
Per cent retention of nitrogen	41.30	43.50	39.76

FIG. 7 URINE CALACIUM CONCENTRATION (mg/dl)
OF GOATS FED THREE RATIONS

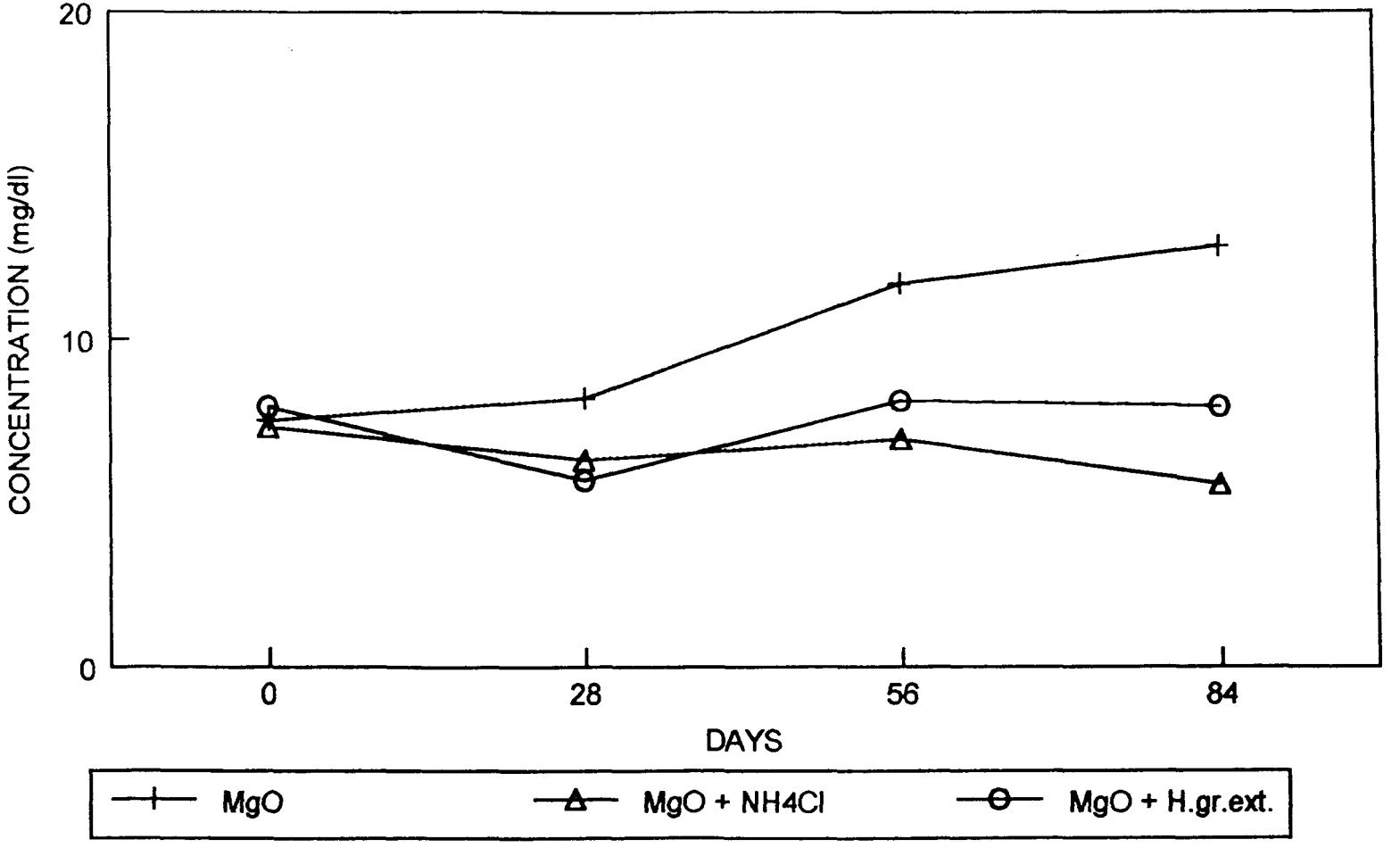


FIG. 8 URINE PHOSPHORUS CONCENTRATION (mg/dl)
OF GOATS FED THREE RATIONS

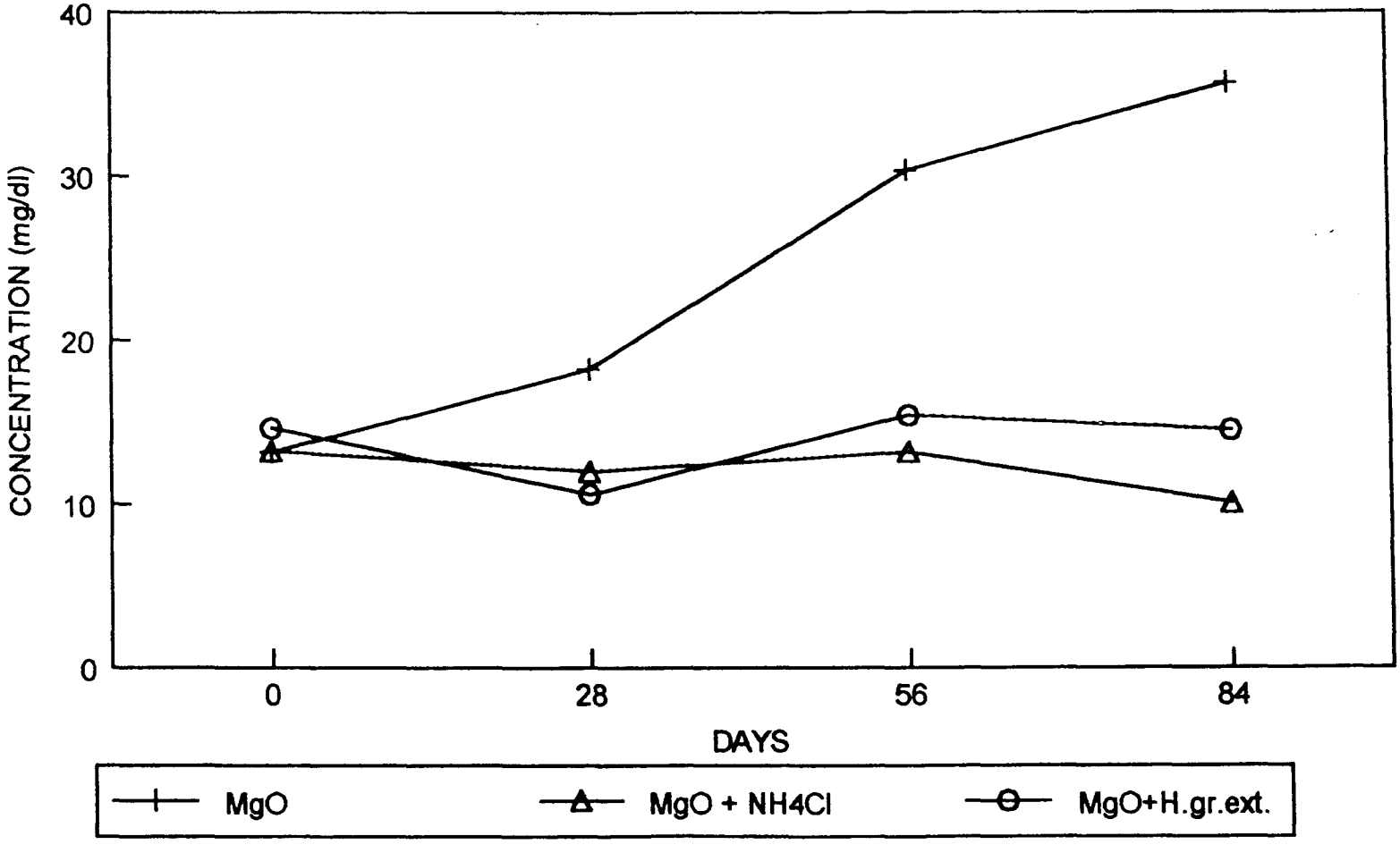


FIG. 9 URINE MAGNESIUM CONCENTRATION (mg/dl)
OF GOATS FED THREE RATIONS

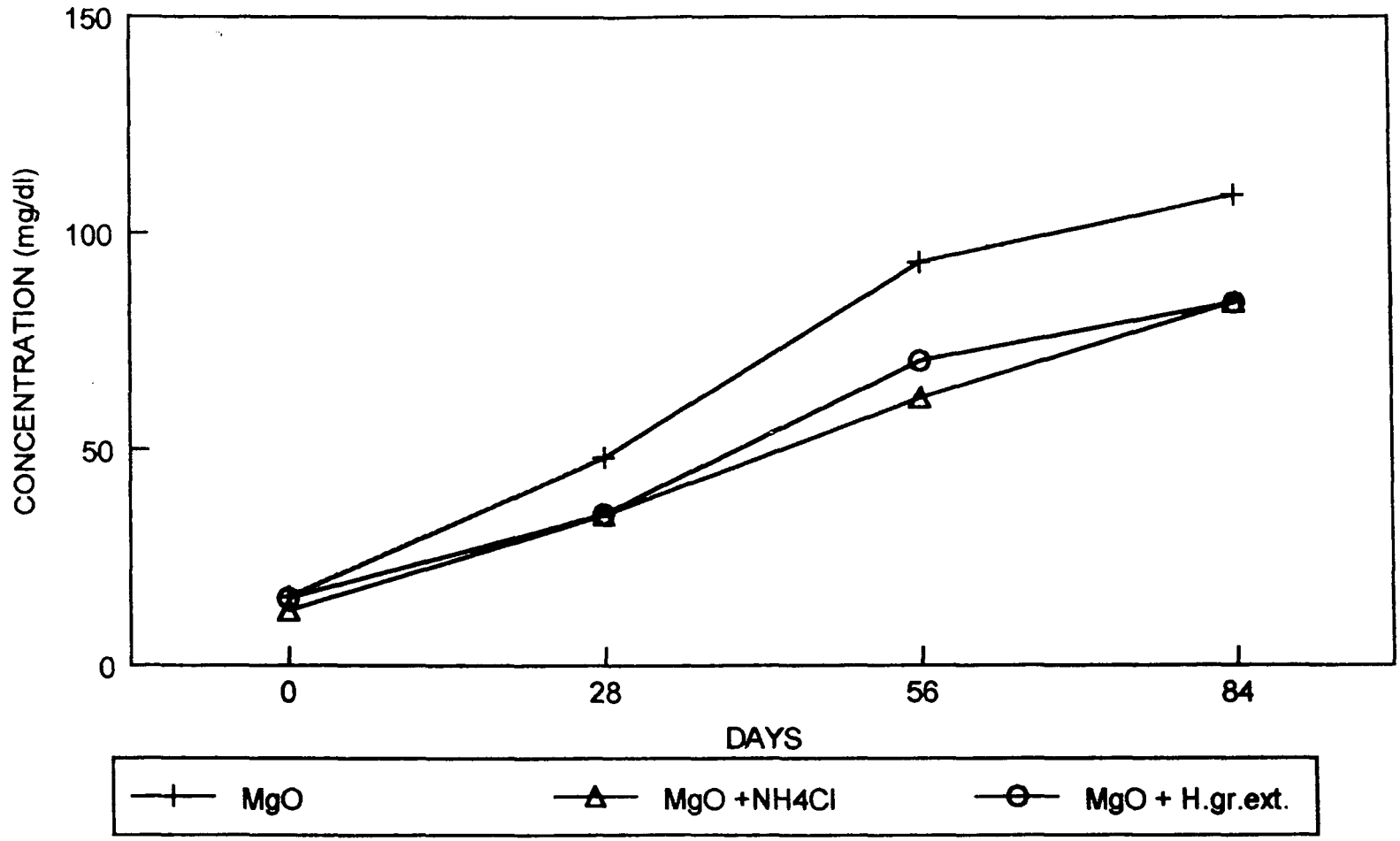


FIG. 10 PER CENT CALCIUM RETENTION OF GOATS MAINTAINED ON THREE RATIONS

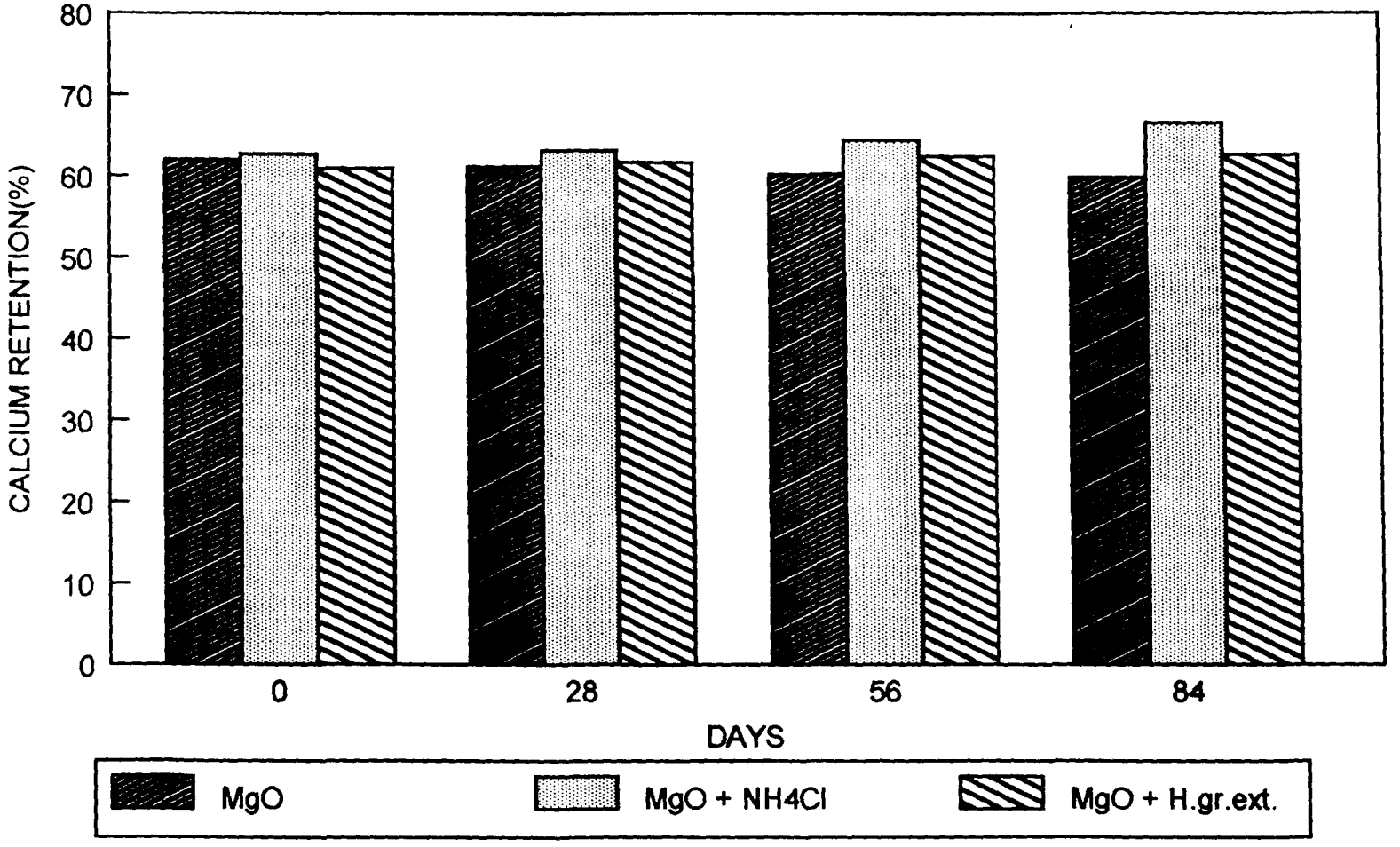


FIG. 11 PER CENT PHOSPHORUS RETENTION OF GOATS MAINTAINED ON THREE RATIONS

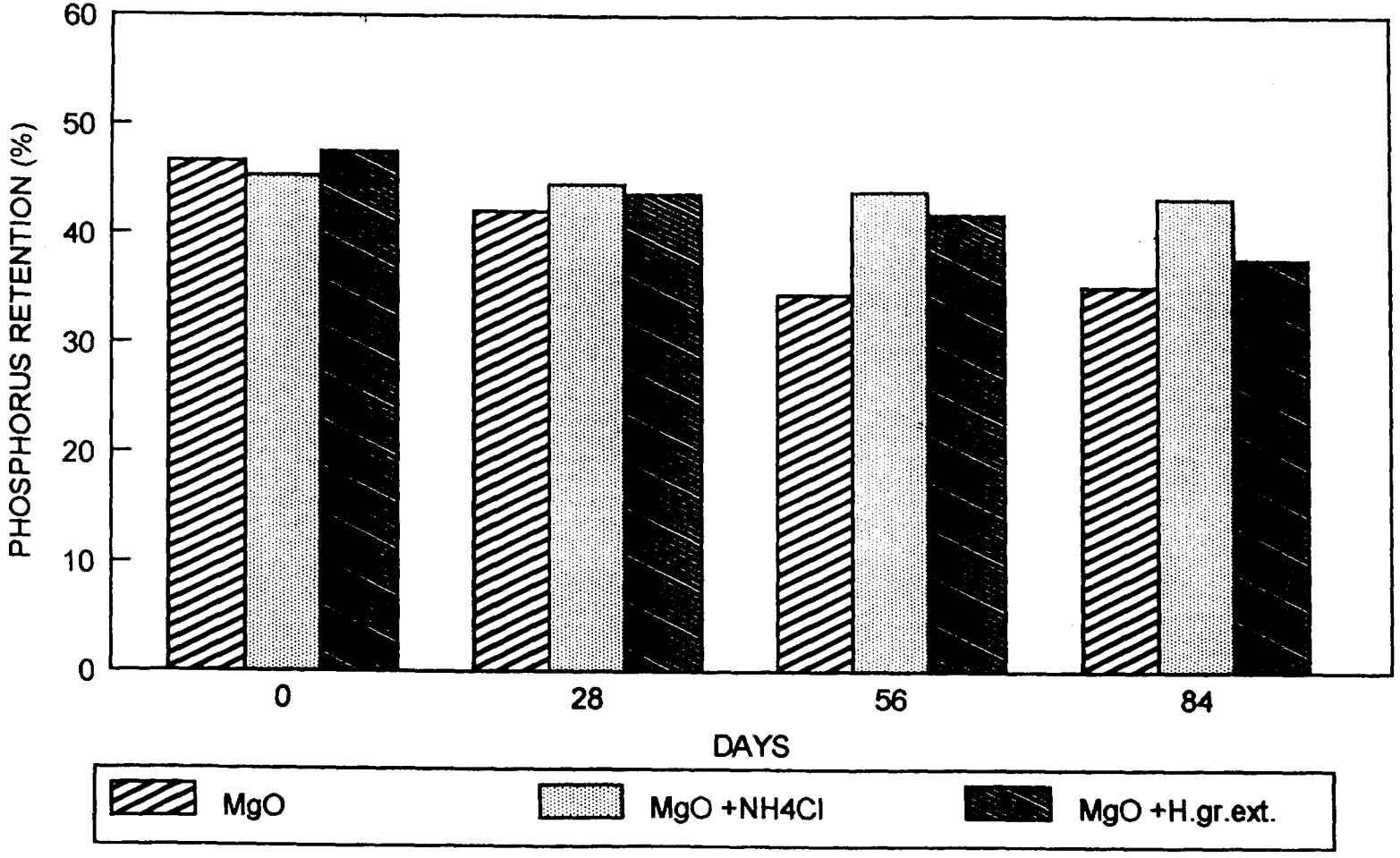


FIG. 12 PER CENT MAGNESIUM RETENTION OF GOATS MAINTAINED ON THREE RATIONS

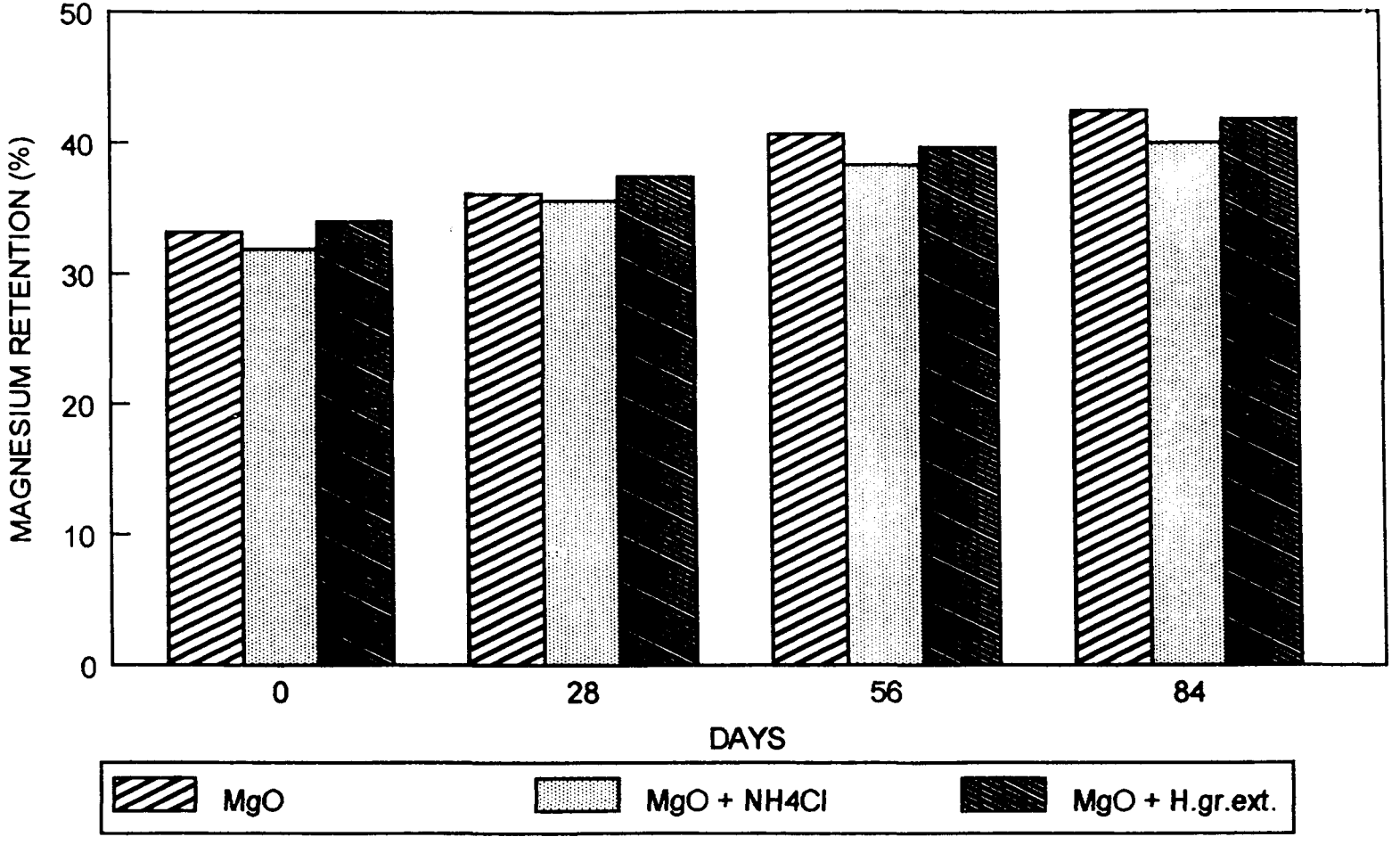


FIG. 13 PER CENT NITROGEN RETENTION OF GOATS MAINTAINED ON THREE RATIONS

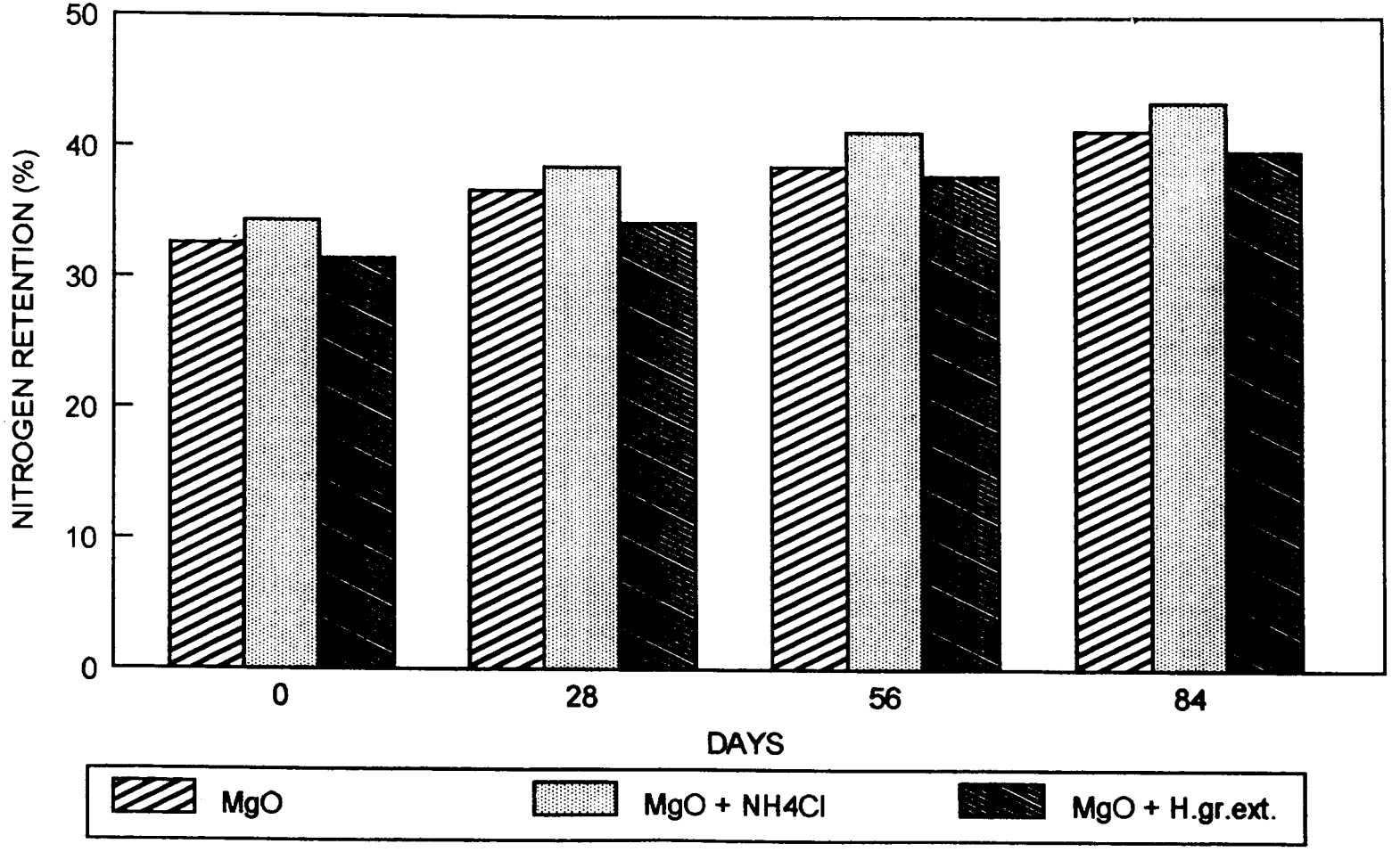


Table 39. Summarised data on urine calcium, phosphorus, magnesium and nitrogen of animals maintained on three experimental rations

Treatments	Ration A containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202%	Ration B containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Ammonium chloride-1%	Ration C containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Horse gram extract 1 lit/animal/day
Number of animals	6	6	6
	Calcium (g/day)		
Initial (0 day)	0.104	0.109	0.108
Final (84th day)	0.191	0.128	0.151
Difference	0.087	0.019	0.043
	Phosphorus (g/day)		
Initial (0 day)	0.182	0.197	0.199
Final (84th day)	0.530	0.231	0.275
Difference	0.348	0.034	0.076
	Magnesium (g/day)		
Initial (0 day)	0.220	0.190	0.212
Final (84th day)	1.617	1.580	1.590
Difference	1.397	1.390	1.378
	Nitrogen (g/day)		
Initial (0 day)	6.865	6.160	6.618
Final (84th day)	11.445	10.953	10.907
Difference	4.580	4.793	4.289

Table 40. Summarised data on faecal calcium, phosphorus, magnesium and nitrogen of animals maintained on three experimental rations

Treatments	Ration A containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202%	Ration B containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Ammonium chloride-1%	Ration C containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Horse gram extract 1 lit/animal/day
Number of animals	6	6	6
	Calcium (g/day)		
Initial (0 day)	3.352	2.925	3.147
Final (84th day)	5.115	4.304	4.472
Difference	1.763	1.379	1.325
	Phosphorus (g/day)		
Initial (0 day)	1.384	1.349	1.218
Final (84th day)	3.740	3.530	3.613
Difference	2.356	2.181	2.395
	Magnesium (g/day)		
Initial (0 day)	1.003	0.977	1.033
Final (84th day)	6.101	6.503	5.973
Difference	5.098	5.526	4.940
	Nitrogen (g/day)		
Initial (0 day)	6.904	6.319	6.854
Final (84th day)	11.610	11.997	11.665
Difference	4.706	5.678	4.811

Table 41. Summarised data on per cent retention of animals maintained on three experimental rations

Treatments	Ration A containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202%	Ration B containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Ammonium chloride-1%	Ration C containing Calcium-1.194% Phosphorus-0.578% Magnesium-1.202% Horse gram extract 1 lit/animal/day
Number of animals	6	6	6
		Calcium (%)	
Initial (0 day)	62.05	62.71	61.04
Final (84th day)	59.85	66.55	62.65
Difference	2.2	3.84	1.61
		Phosphorus (%)	
Initial (0 day)	46.64	45.28	47.48
Final (84th day)	35.24	43.24	37.72
Difference	11.40	2.56	9.76
		Magnesium (%)	
Initial (0 day)	32.20	31.86	34.00
Final (84th day)	42.49	40.01	41.80
Difference	9.7	8.15	7.8
		Nitrogen (%)	
Initial (0 day)	32.60	34.35	31.43
Final (84th day)	41.30	43.50	39.76
Difference	8.7	9.15	8.33

Table 42. Analysis of variance - Urine calcium, phosphorus, magnesium and nitrogen

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
		Calcium		
Between	2	0.294	0.147	4.109*
Within	15	0.536	0.036	
Total	17	0.830		
		Phosphorus		
Between	2	0.307	0.153	19.634**
Within	15	0.117	0.008	
Total	17	0.424		
		Magnesium		
Between	2	0.001	0.001	0.019NS
Within	15	0.475	0.032	
Total	17	0.476		
		Nitrogen		
Between	2	1.364	0.682	0.503NS
Within	15	20.347	1.356	
Total	17	21.710		

* Significant at 5% level
** Significant at 1% level
NS - Not significant

Table 43. Analysis of variance - Faecal calcium, phosphorus, magnesium and nitrogen

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
		Calcium		
Between	2	1.126	0.563	1.357NS
Within	15	6.225	0.415	
Total	17	7.351		
		Phosphorus		
Between	2	0.079	0.039	0.217NS
Within	15	2.711	0.181	
Total	17	2.789		
		Magnesium		
Between	2	1.759	0.879	1.322NS
Within	15	9.979	0.665	
Total	17	11.737		
		Nitrogen		
Between	2	4.98	2.49	2.297NS
Within	15	16.26	1.084	
Total	17	21.245		

NS - Not significant

Table 44. Analysis of variance - Per cent retention of calcium, phosphorus, magnesium and nitrogen

Source	Degrees of freedom	Sum of squares	Mean sum of squares	F-value
		Calcium		
Between	2	33.955	16.977	6.822**
Within	15	37.327	2.488	
Total	17	71.281		
		Phosphorus		
Between	2	200.198	100.099	5.078*
Within	15	295.664	19.711	
Total	17	495.862		
		Magnesium		
Between	2	7.810	3.905	1.238NS
Within	15	47.303	3.154	
Total	17	55.112		
		Nitrogen		
Between	2	4.440	2.220	0.464NS
Within	15	71.695	4.780	
Total	17	76.135		

* Significant at 5% level
** Significant at 1% level
NS - Not significant

4.5 Clinical observation

During the 84 days of experimental period, any clinical signs of obstructive urolithiasis like abdominal pain with kicking at the belly, treading with hindfeet and swishing of the tail, repeated twitching of penis sufficient to shake the prepuce, strenuous efforts to urinate accompanied by straining, grunting and grating of the teeth with passage of only few drops of blood stained urine or in the case of incomplete obstruction, voiding small amount of blood stained urine frequently followed by complete blockage or in extreme cases, distention of abdomen and toxemia due to rupture of bladder and mortality due to incidence of urolithiasis (Blood and Radostits, 1989) were not observed in any of the goats maintained on three experimental rations.

4.6 Examination of urine

Severe crystalluria (Plates 3 and 4) was exhibited in group I, but intermittent crystalluria at varying intensity was seen in group III and the crystalluria was absent in group II. The pH of the urine was 8.0, 8.3 and 8.4 in groups II, III and I respectively. Also, group II and III had increase in average urine volume compared to group I (1.866.93, 1774.74 and 1412.9 ml per day respectively).

4.7 Postmortem findings

At the end of the experimental period, all goats were slaughtered and the urinary organs were subjected to detailed post-mortem and histopathological examination. Post-mortem examination the urinary organs disclose the following findings and are depicted in Plates 5 to 9.

Group I

Kidneys were moderately enlarged. White patches and focal areas of haemorrhage of varying intensity were seen on the cortex of the kidney. Pelvis of the kidney had numerous visible sand like rudimentary calculi. The bladder had thickened mucosal layer with hyperemic changes. Focal haemorrhages and erosions were noticed on the mucosal surface of the bladder. No calculi were recovered from ureter, bladder or from the urethra. But near to neck of the bladder, urethra had shown hyperemic changes.

Group II

There was slight enlargement of kidneys. Bladder and urethra did not show any visible changes. No visible calculi materials were detected in any part of urinary tract.

Group III

Kidney had slight enlargement with focal areas of haemorrhages and few greyish white patches on cortex. Few rudimentary calculi were recovered from the pelvis of the kidney. There was thickening of the mucosa of the bladder with focal areas of haemorrhages. No calculi were detected in bladder, ureter or in the urethra, but hyperemic changes seen in urethra near to the neck of bladder.

4.8 Histopathological findings

Histopathological examination of the kidney and bladder discloses the following findings and are depicted in Plates 10 to 22.

Group I

Kidney showed severe destruction of tubular epithelium with engorged blood vessels and thickened vessel walls. There were intense inflammatory reaction with infiltration of lymphocytes and macrophages in the interstitial areas of the kidney. There was presence of numerous microcalculi of varying stages of development seen in the tubular lumen. Tubules showed cystic dilatation with pink stained hyalinised proteinaceous mass in the lumen. Bladder epithelial cells were enlarged and sloughed out in some places.

Group II

Tubular epithelial cells undergoing hyalinisation. Some tubules showed hyalinised pink stained protein casts and focal areas of inflammation with infiltration of lymphocytes and macrophages and proliferation of fibrous tissue in between the tubules. No microcalculi were detected in the tubules.

Group III

Kidney had dilated and engorged blood vessels. There were degeneration and necrosis of tubular epithelium with presence of numerous microcalculi in the lumen of the tubules. Hyalinised pink stained proteinaceous casts seen in many dilated tubules. Focal areas of inflammation with infiltration of lymphocytes and macrophages in the interstitial cells.

Spot tests (Winer, 1959) were carried out for the identification of common crystalloids in the urinary calculi. The results indicate that the calculi recovered during the present study composed of magnesium, phosphate and ammonium.

Plate 3. Urine crystals collected from goats maintained on ration A (fresh mount) X100

Plate 4. Urine crystals collected from goats maintained on ration A (fresh mount) X250

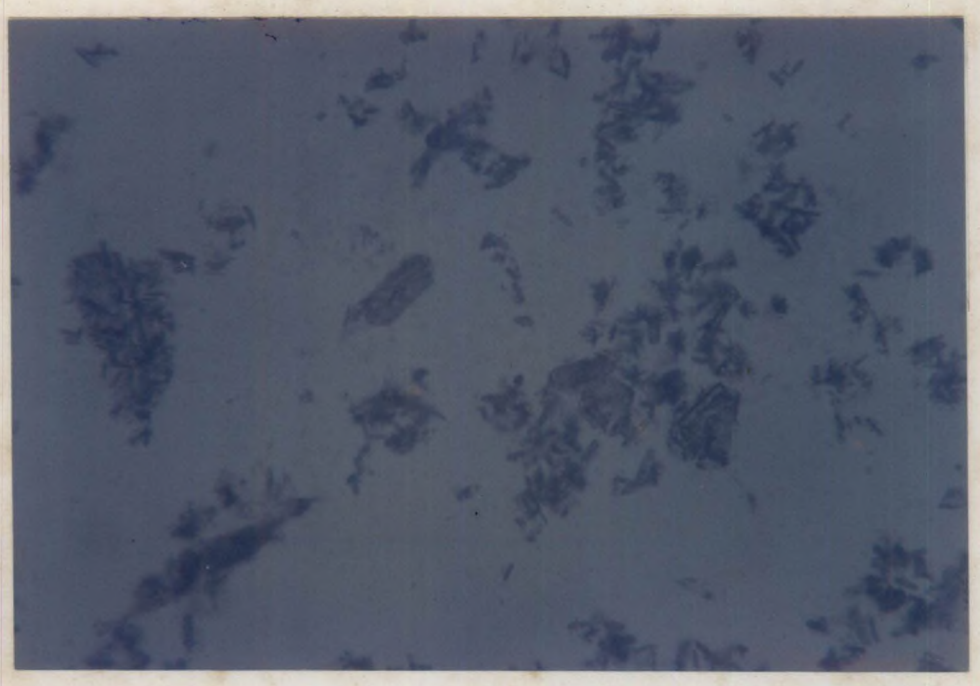
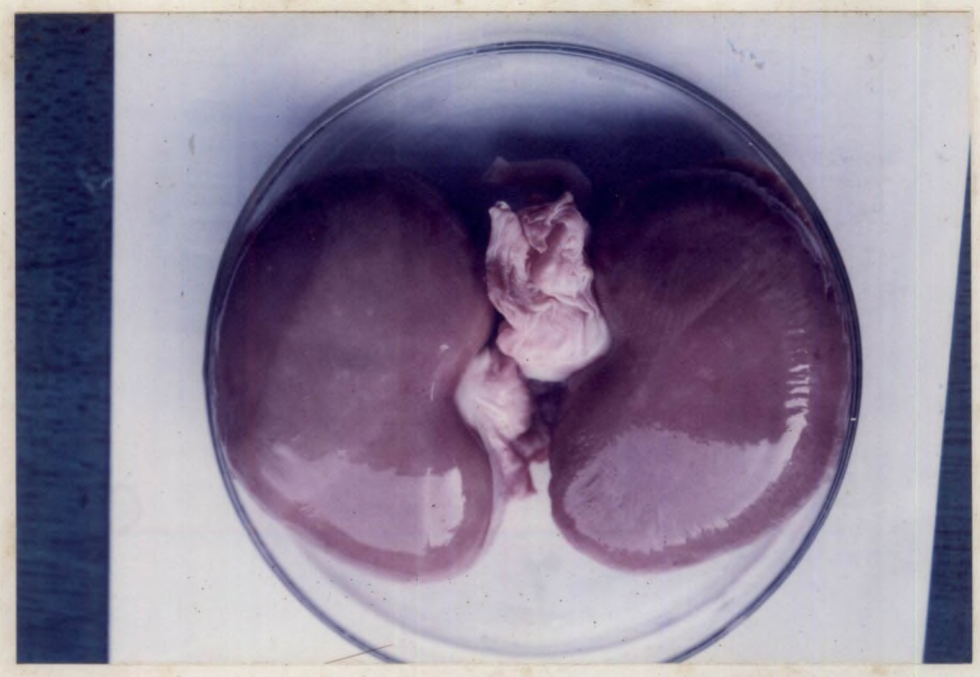


Plate 5. Small greyish white areas of degeneration on the cortex of the kidney from group I

Plate 6. Numerous visible sandlike calculi in the kidney from group I



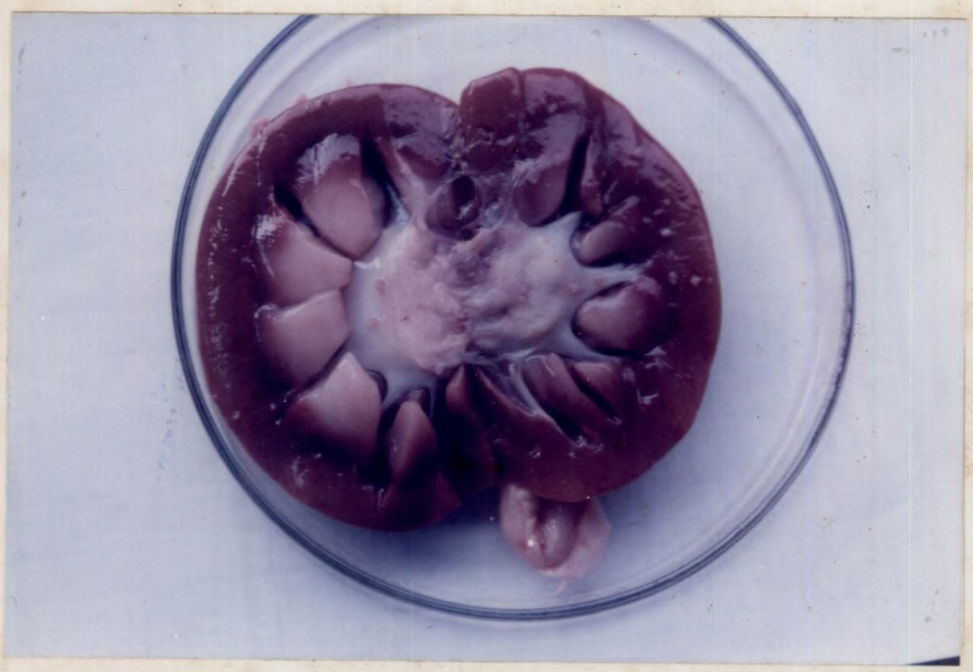
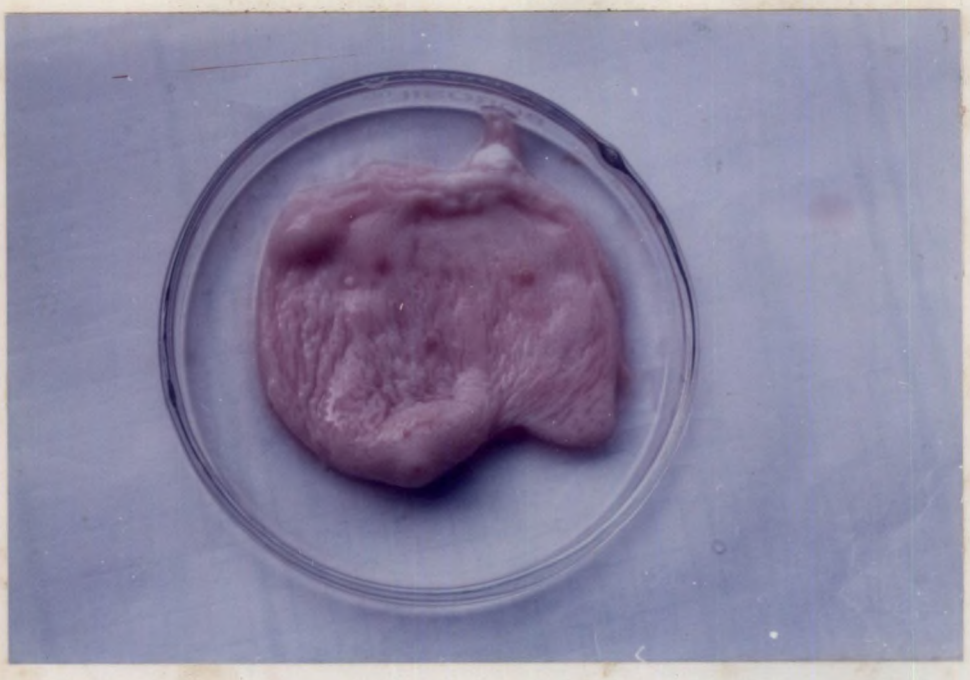


Plate 7. Erosions on the mucosa of the bladder from group I

Plate 8. Hyperemia in the mucosa of urethra from groups I and III



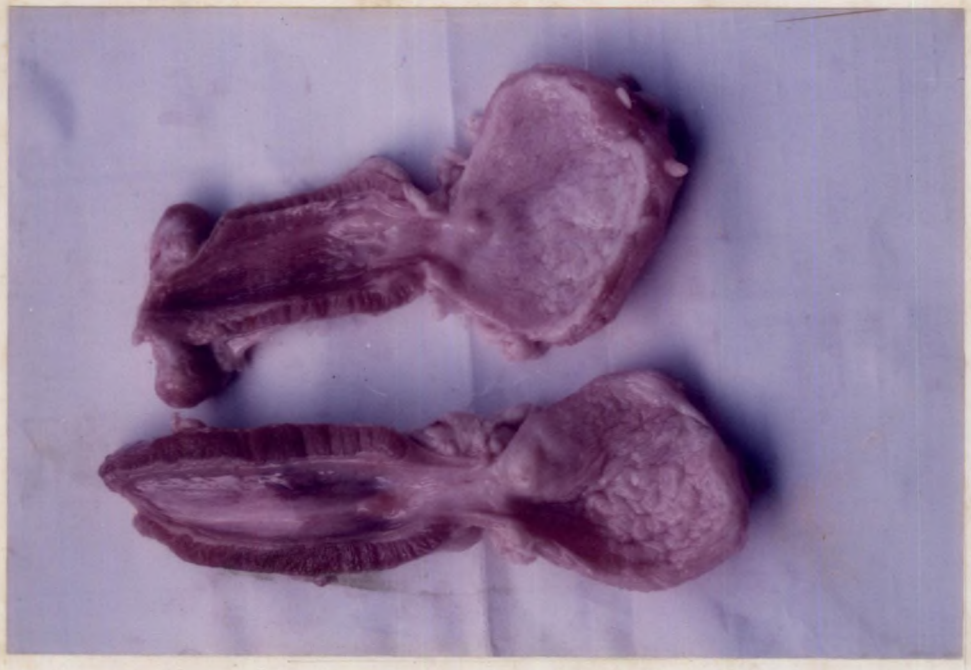
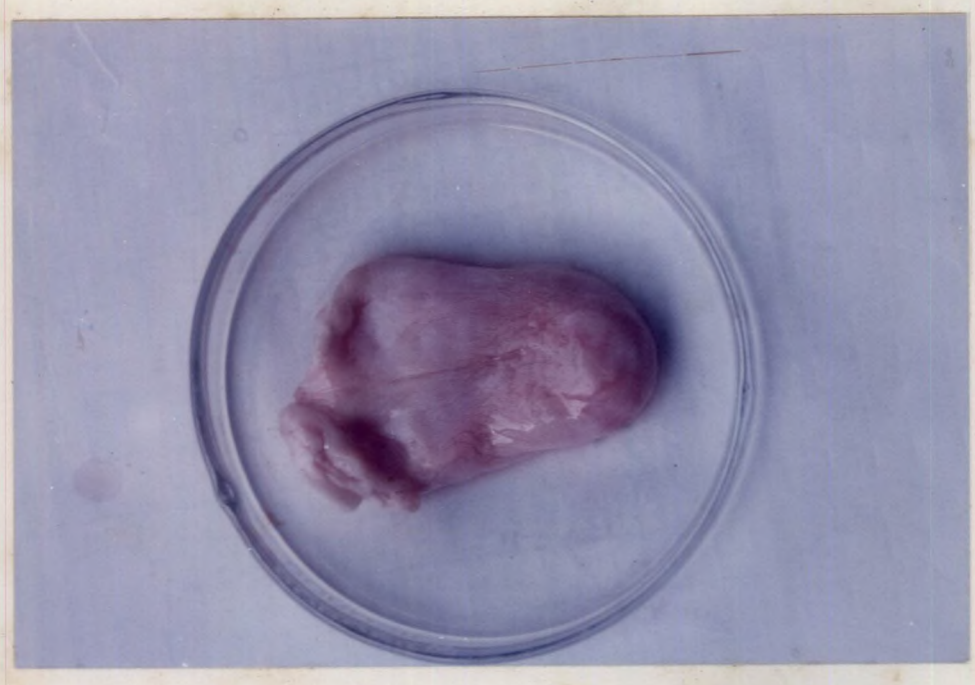


Plate 9. **Hyperemia on mucosa of bladder from group III**

Plate 10. **Presence of microcalculi in the tubular lumen, thickening of vessel wall, dilated tubules - H&E X160**



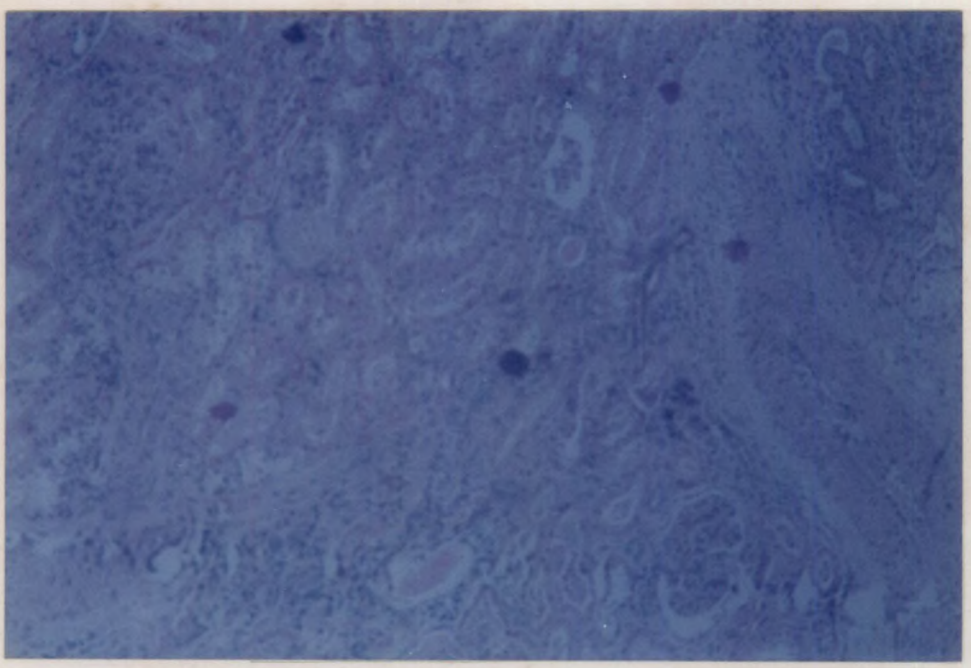
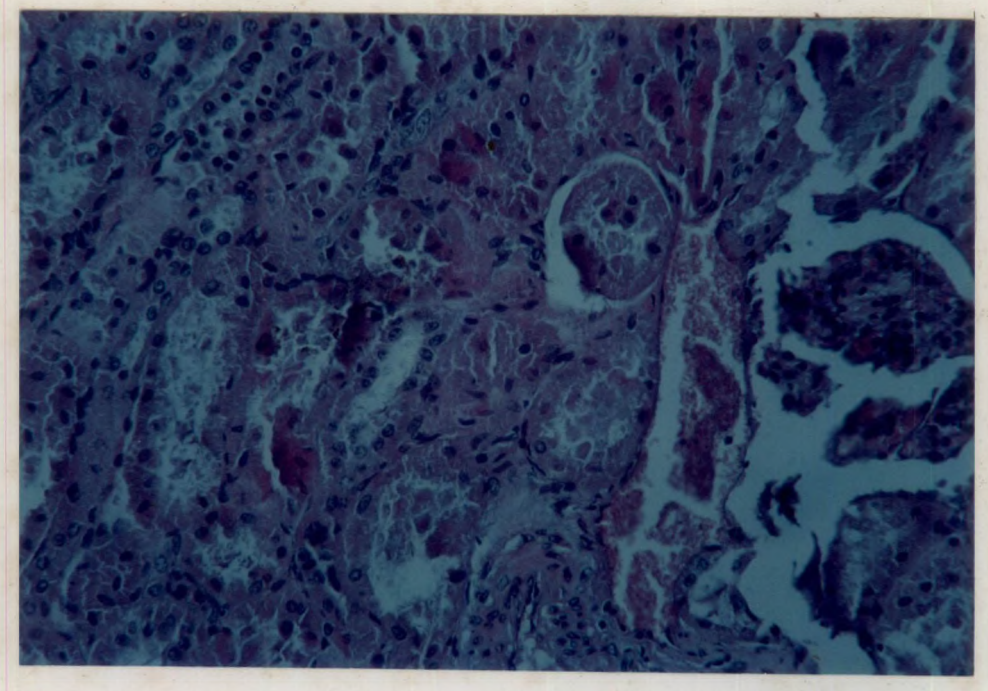


Plate 11. Severely engorged blood vessels, destruction of tubular epithelium - H&E X450

Plate 12. Presence of microcalculi, destruction of tubular epithelium - H&E X450



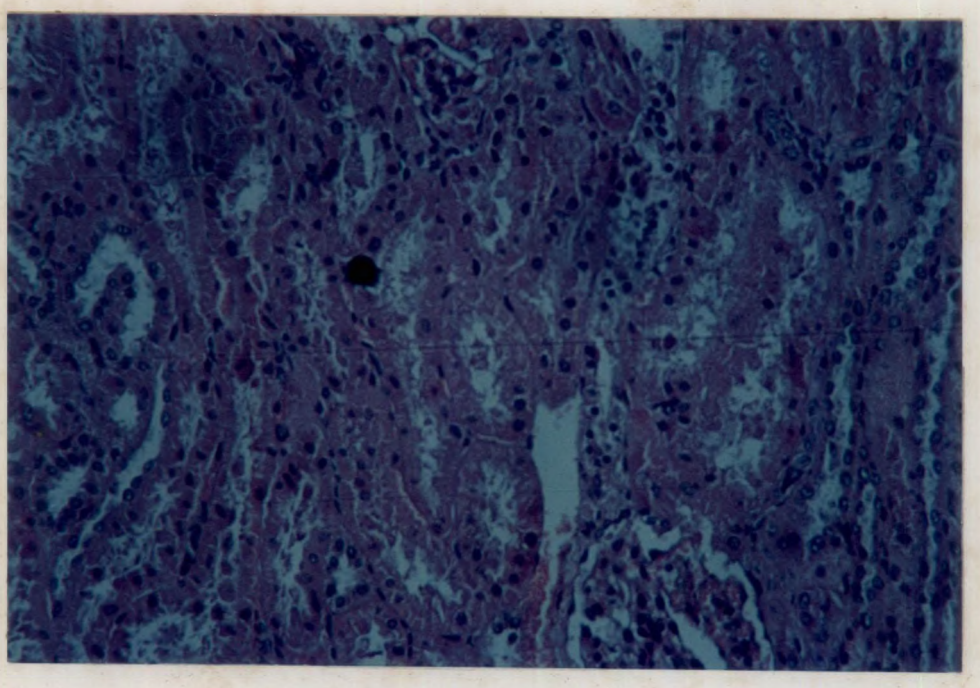
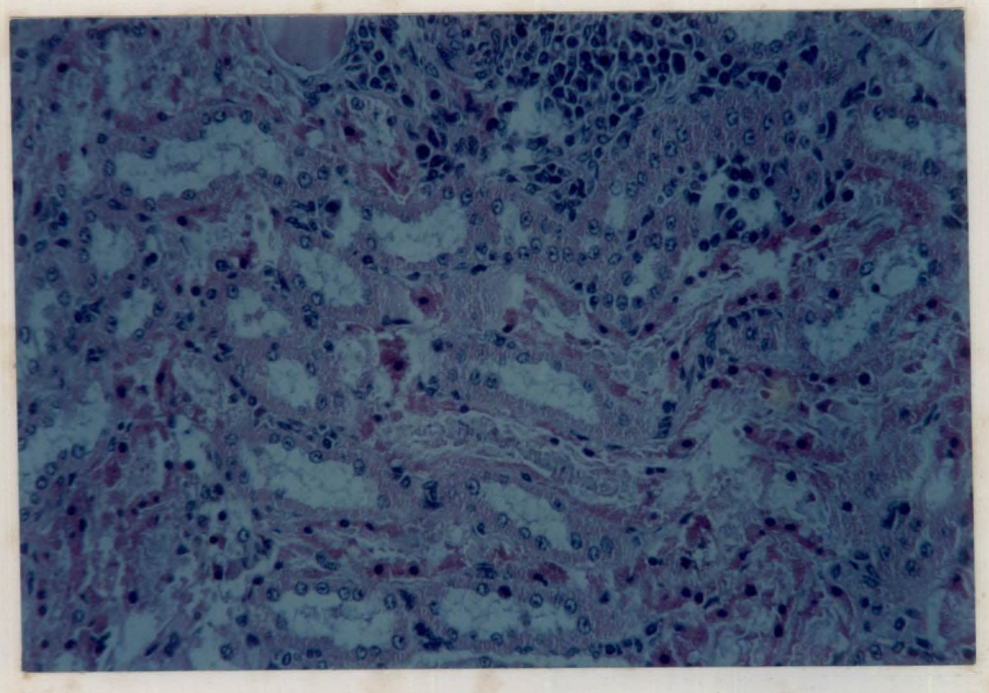


Plate 13. **Presence of inflammatory cells, dilated tubules containing cellular debris - H&E X450**

Plate 14. **Destruction of the tubules, presence of developing microcalculi, hyalinised pink stained proteinacious mass - H&E X450**



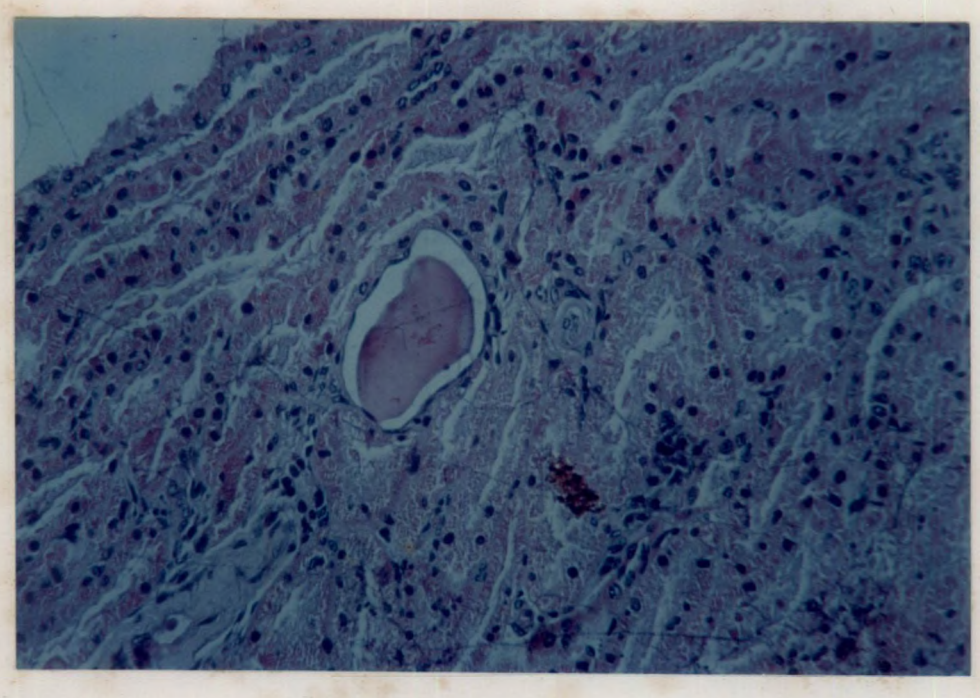
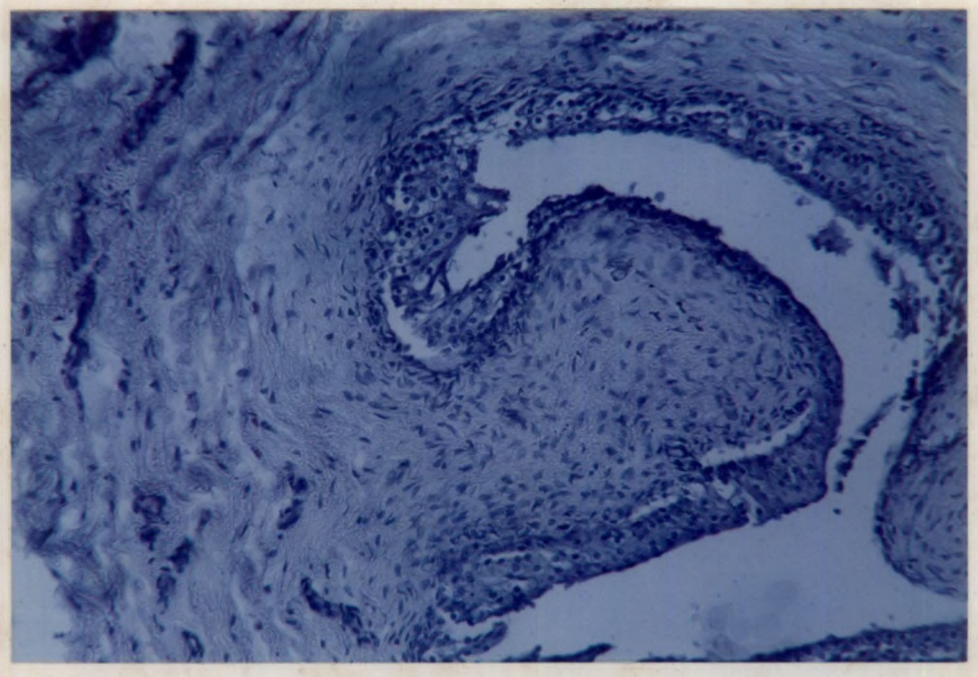


Plate 15. Thickened mucosa of the bladder, focal sloughing of mucosa - H&E X450

Plate 16. Focal areas of inflammation with infiltration of lymphocytes and macrophages - H&E X450



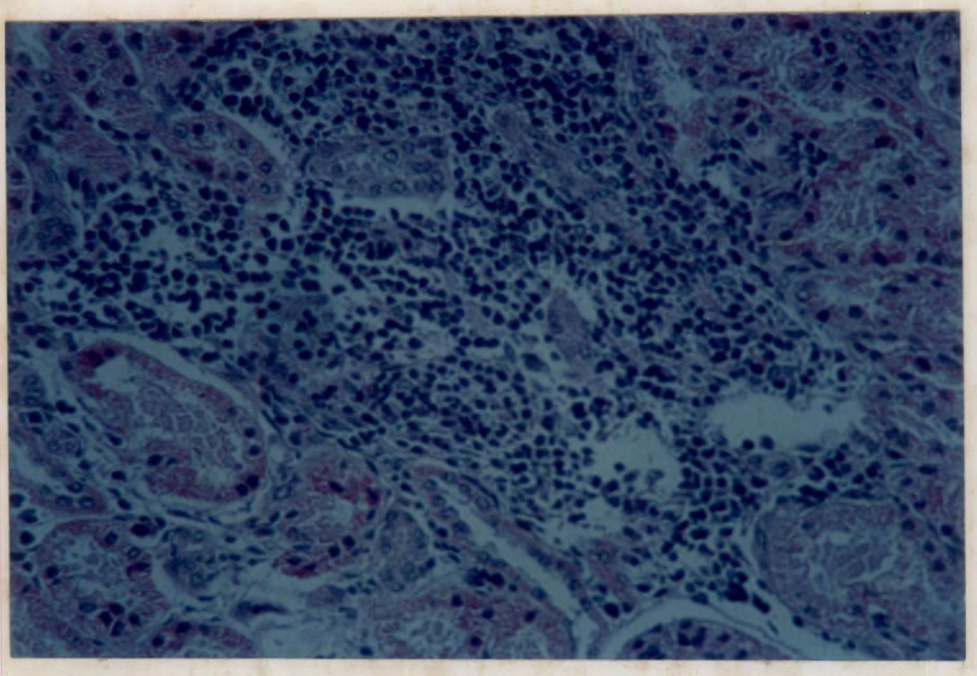
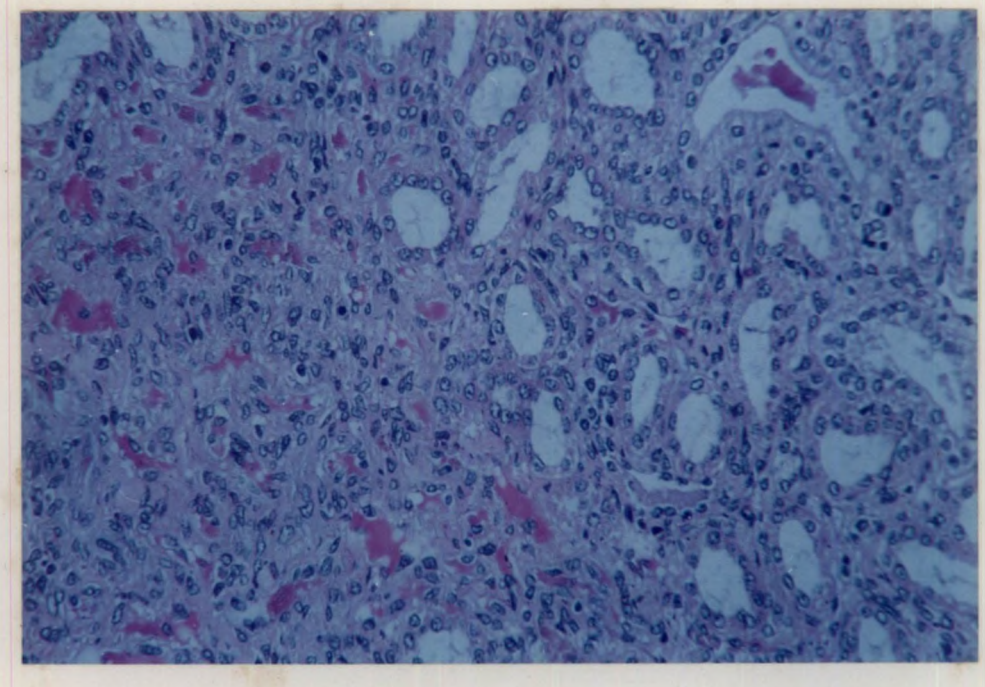


Plate 17. **Dilated tubules, presence of hyalinised pink stained proteinacious mass, fibrosis - H&E X450**

Plate 18. **Dilated tubules, developing calculi in the lumen of tubule, destruction of tubular epithelium - H&E X450**



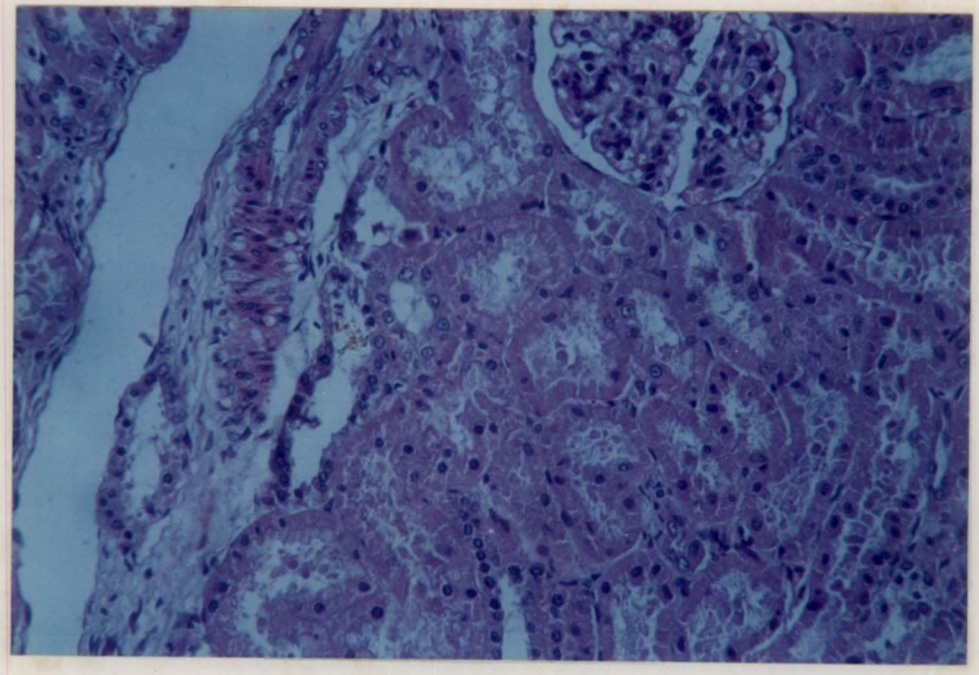
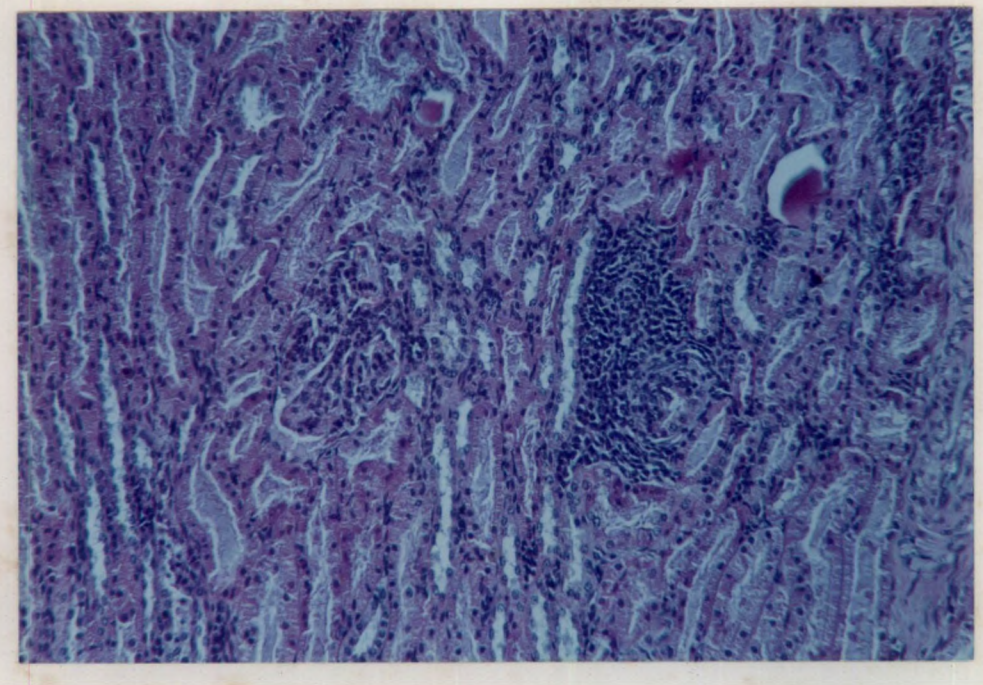


Plate 19. **Presence of an inflammatory foci, pink stained hyaline mass in the tubule - H&E X250**

Plate 20. **Focal areas of inflammation, dilated tubule, presence of microcalculi in the lumen of the tubule - H&E X 250**



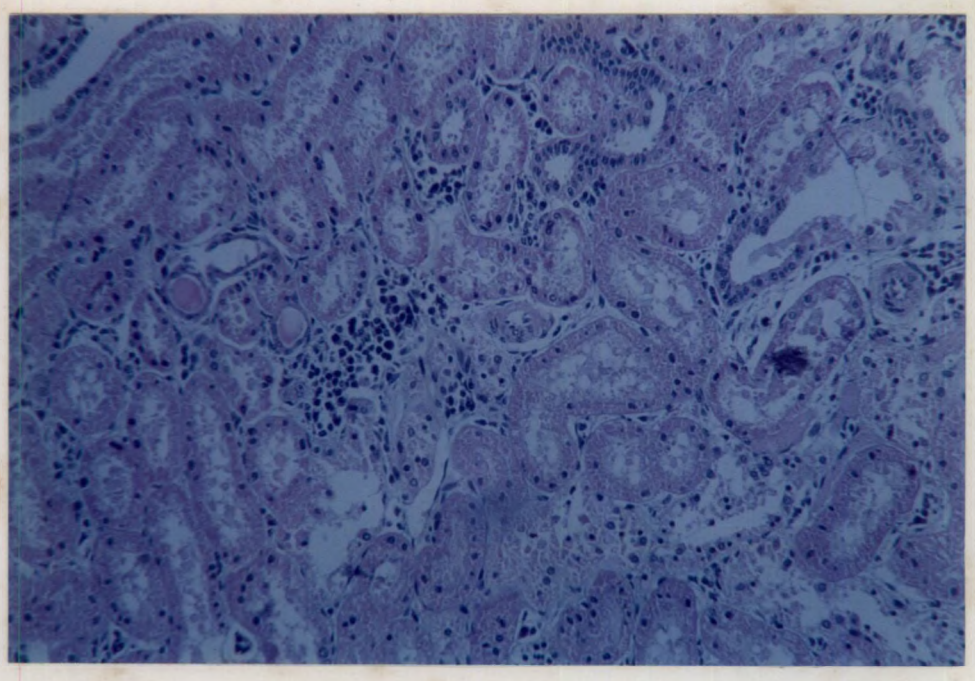
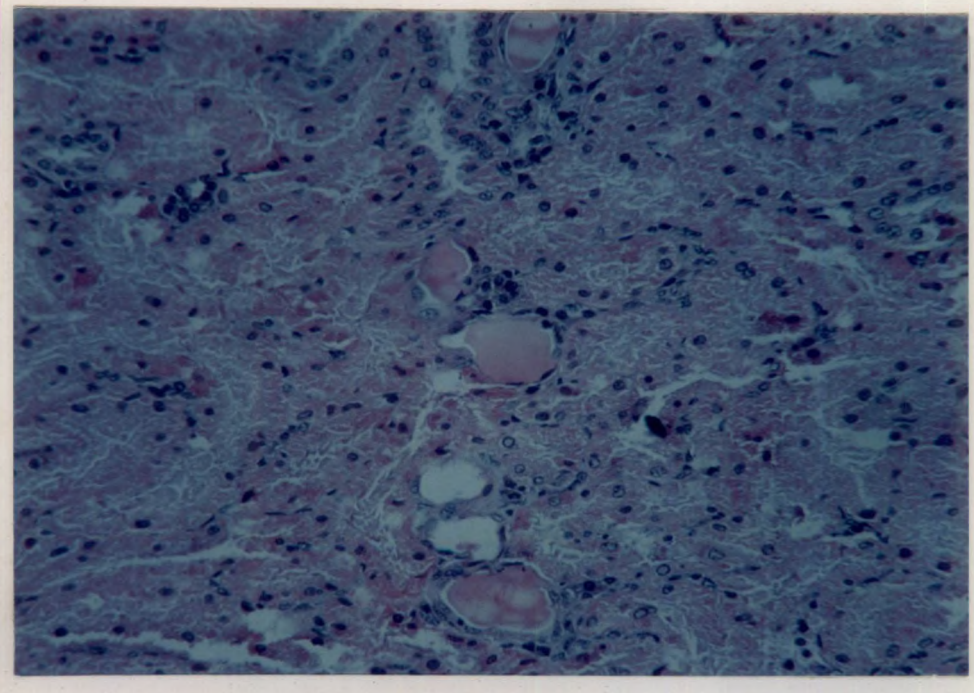
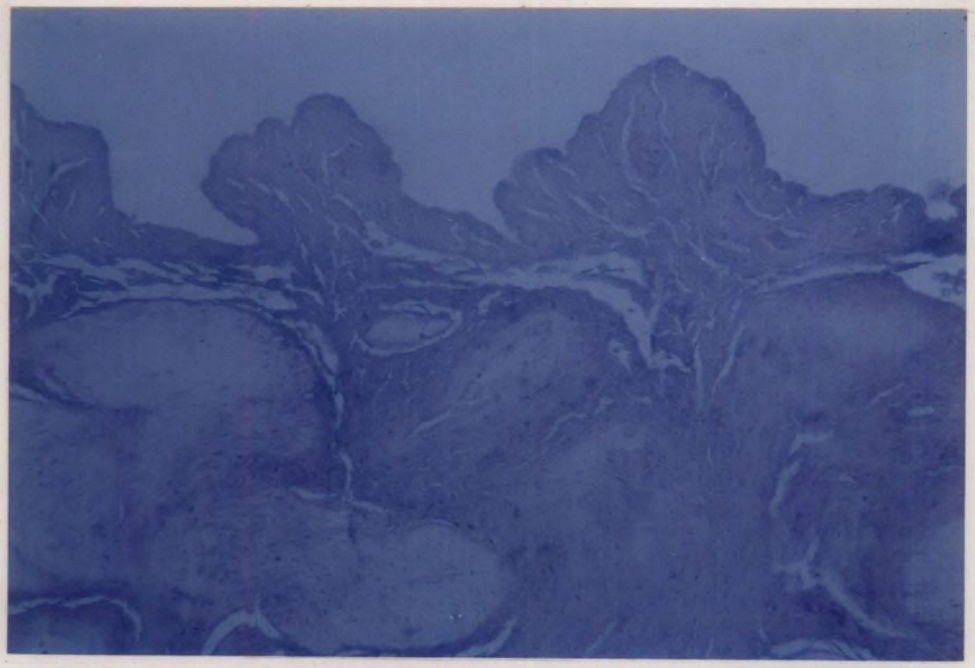


Plate 21. **Destruction of the tubules, presence of pink stained hyalinised mass in the tubule - H&E X250**

Plate 22. **Thickened mucosa of the bladder - H&E X160**





Discussion

5. DISCUSSION

The results obtained during the course of present investigation are discussed under separate heads.

5.1 Body weight gain

The data presented in Table 1, summarised in Table 5, represented by Figure 1 indicate that goats maintained on ration A containing 1.194 per cent calcium,0.578 per cent phosphorus and 1.202 per cent magnesium which is being the calculogenic ration constituting the control, ration B being the calculogenic ration supplemented with ammonium chloride to the extent of one per cent of the total ration and ration C being the calculogenic ration supplemented with horse gram extract to the extent of one litre per animal per day exhibited an appreciable increase in body weight gain during the experimental period of 84 days, the average daily gain of the animals maintained on three rations viz., rations A, B and C being 61.9 g, 62.98 g and 61.55 g with an average cumulative body weight gain of 5.20, 5.29 and 5.17kg respectively. Statistical analysis of the data presented in Tables 3 and 4 indicated no significant difference in growth rate between the animals maintained on the respective rations. However, a

trend towards better weight gain was observed in kids maintained on ration B, though not statistically significant.

James (1968) from their investigations on experimental production of urolithiasis in goats observed an average growth rate of 64.9 to 71.7 g per day and in another experiment, James (1973) observed a growth rate varying between 70 to 72 g fed with various levels of calcium, phosphorus and magnesium and concluded that neither supplementation of calcium nor magnesium had any significant influence on growth rate.

Mercy *et al.* (1981) from their study to assess the nutrient requirement of Alpine-Malabari crossbred goats observed an average daily gain of 63.7 to 67.7 g per day in three groups of kids maintained on different plane of nutrition.

From a perusal of results obtained on growth, it can be seen that the overall daily gain of the animals observed in the present study were comparatively lower than those reported by other workers (James, 1968 and 1973; Mercy *et al.*, 1981) which may be attributed to the goats of 9 to 12 months old being utilized for the present investigation. The results also tend to suggest that neither supplementation of ammonium

chloride nor horse gram extract along with the calculogenic ration had any profound effect on the growth of animals during the experimental period.

5.2 Dry matter consumption

Data presented in Table 5, consolidated in Table 8 and statistical analysis of the data set out in Table 9 on feed intake of goats maintained on the three respective rations reveal no significant difference between the groups. The average daily dry matter intake (g), recorded for the goats maintained under the groups I, II and III being 928.57, 917.98 and 901.38 and when expressed on percentage body weight being 3.71, 3.64 and 3.62 respectively indicating that neither supplementation of ammonium chloride to the extent of one per cent nor horse gram extract to the extent of one litre per animal per day have any influence on dry matter consumption.

James (1968 and 1973) reported that neither supplementation of calcium to the extent of 1.7 per cent nor supplementation of both 0.6 and 1.102 per cent level of magnesium evince any influence on dry matter consumption.

Mercy *et al.* (1981) from their studies on nutrient requirement of Alpine-Malabari kids for growth reported an intake of 3.1, 3.5 and 3.6 kg of dry matter per 100 kg body weight for three groups of kids maintained on low, medium and high plane of nutrition respectively.

Gangadevi (1981) observed a dry matter consumption on percentage body weight was 3.8, 3.6, 2.7 and 3.4 respectively for kids in groups I, II, III and IV maintained on 16, 18, 20 and 22 per cent of crude protein in the ration.

The result obtained during the course of present investigation on dry matter consumption is in keeping with the findings of above authors (James 1978, 1973; Mercy *et al.*, 1981 and Gangadevi, 1981).

5.3 Feed efficiency

It can be seen from the data presented on feed efficiency in Table 6 represented by Figure 2, consolidated data in Table 8, and the statistical analysis in Table 10 of animals maintained on respective rations that the efficiency of feed utilization were 14.58, 14.69 and 15.02 in that descending order for the goats maintained on the groups II, III and I respectively. However, no significant difference exist on

feed efficiency between the groups. On scrutiny of the data, it can be further perceived that there is a slight increase in feed efficiency in animals maintained on ration B supplemented with ammonium chloride to the extent of one per cent.

James (1973) observed a feed efficiency of 10.32 in goats maintained on high magnesium calculogenic ration. Thomas *et al.* (1976) reported values ranging from 6.18 to 7.1 for Jamnapari-Malabari crossbred kids from their feeding experiments on evaluation of nutritive value of raintree fruit meal for growth in kids. For Saanen-Malabari crossed kids maintained on three rations with either rubber seed cake or tea waste as one of the ingredient, James (1978) reported feed efficiency values ranging from 5.5 to 6.8.

Mercy *et al.* (1981) during the course of their study to determine nutrient requirement of kids, obtained values ranging from 7 to 7.9 for Alpine-Malabari crossed kids. Deepa Ananth (1998) from her studies on nutritive evaluation of complete ration for growth in kids recorded cumulative feed efficiency values of 9.091, 9.331 and 9.670 for the different rations under investigation.

The values obtained during the course of the present investigation on efficiency of feed conversion for goats

receiving different experimental rations were higher than those reported by the above authors (James, 1973, Thomas *et al.*, 1976, James, 1978, Mercy *et al.*, 1981 and Deepa Ananth, 1998) probably due to 9 to 12 months old goatsbeing used for the present investigation.

A positive trend observed on feed efficiency of animals maintained on supplemental ammonium chloride group is in keeping with the observations made by Crookshank (1970) who observed a significant increase in feed efficiency in lambs fed with calculogenic ration supplemented with ammonium chloride to the extent of 0.5 per cent in the ration.

5.4 Protein efficiency values

The protein efficiency values registered with regard to respective dietary treatments presented in Table 4, depicted in Figure 3 and that summarised in Table 8, were 2.69, 2.60 and 2.62 for the goats maintained on rations A, B and C respectively and no significant difference could be seen among the groups on statistical analysis of the data presented in Table 11, indicating that neither supplementation of ammonium chloride nor horse gram extract had influenced the efficiency of protein utilisation of the animals under experimentation. On scrutiny of the data, it can be further observed that the

protein efficiency values were slightly better in ammonium chloride supplemented group (group II) when compared to that of control calculogenic (group I) and horse gram supplemented group (group III).

James (1973) reported that a ration having various levels of calcium, phosphorus and magnesium when fed to goats had obtained protein efficiency values ranging from 1.62 to 1.66 which is slightly lower than the values obtained during the course of the present investigation. Similar lower protein efficiency values were also reported by Deepa Ananth (1998) from her work on nutritive evaluation of complete feed for growth in kids.

5.5 Haematological values

5.5.1 Total erythrocyte, total leucocyte, haemoglobin and total protein

Data on haematological parameters like total erythrocyte count, total leucocyte count, haemoglobin and plasma protein concentrations in blood of goats maintained on three dietary regimes presented in Tables 12, 13, 14 and 15 and that summarised in Table 16 with their statistical analysis presented in Table 17 clearly indicate that all the goats

showed the values within the normal range, irrespective of the dietary treatments. The statistical analysis also indicated that administration of ammonium chloride to the extent of one per cent in a calculogenic ration or horse gram extract to the extent of one litre per animal per day had not induced any significant influence on haematological parameters.

Average values with respect to total erythrocyte count ($10^6/mm^3$) obtained during the course of present investigation were ranging from 12.41 to 13.25, 12.25 to 13.34 and 12.04 to 13.97 respectively for the animals maintained on groups I, II and III. Total leucocyte count ($10^3/mm^3$) recorded during the present study ranging from 10.54 to 11.94, 11.40 to 12.70 and 11.10 to 11.76 and average values recorded for haemoglobin (g/dl) for the animals maintained on the three groups being 8.27 to 8.63, 8.04 to 8.57 and 8.23 to 8.53 respectively and those for plasma protein (g/dl) the average values ranging from 7.49 to 8.09, 7.26 to 7.83 and 7.02 to 7.79 respectively for the groups of animals maintained on ration A, B and C. The haemotological values obtained during the course of present investigation fall in accordance with that reported by James (1973), Gangadevi (1981), Mercy (1979), Shyama (1994) and Deepa Ananth (1998), showing that the animals maintained on normal nutritional status.

5.5.2 Calcium, phosphorus and magnesium concentrations in blood

5.5.2.1 Calcium

The serum calcium concentration (mg/dl) values of the animals maintained on three groups obtained on monthly intervals are presented in Table 18, represented by Figure 4 and that summarised in Table 21, recorded 8.67, 9.29 and 8.99 mg/dl respectively and on statistical analysis of the data presented in Table 22, disclose a significant ($P<0.01$) reduction in serum calcium concentration in group I receiving ration A when compared to that of groups II and III receiving rations B and C respectively, as can be appreciated from a significant difference ($P<0.01$) between the groups I versus II and I versus III. However, no significant difference could be noticed between the groups II and III indicating that the animals maintained on high magnesium calculogenic ration had a tendency to decrease the absorption of dietary calcium. On scrutiny of the data on serum calcium concentration, it can be further perceived that there is no change in calcium concentration level in animals maintained on ammonium chloride supplemented group as well as in horse gram extract supplemented group indicating that the absorption of calcium has not been altered due to dietary treatments. In other

words, there is an indication that supplemental ammonium chloride or supplemental horse gram extract have counteracted the influence of high dietary magnesium over calcium absorption.

Packett and Hauschild (1964) observed that on feeding rations containing 0.26 and 0.30 per cent calcium, 0.24 and 0.30 per cent phosphorus and 0.21 and 0.22 per cent of magnesium produced urolithiasis in lambs which had reduction in serum calcium (mg/dl) level from 8.79 and 8.81 to 8.46 and 8.43 at 11th week of experiment.

James (1968) reported that goats fed ration having 0.386 per cent calcium 0.267 per cent phosphorus and 0.6 per cent magnesium did not have any influence on serum calcium level. However, James (1973) reported an identical ration used in the present study when fed to goats caused reduction in serum calcium concentrations (mg/dl) from 12.6 to 12.2 during the course of the experiment.

Kallfelz *et al.* (1986) reported that feeding high magnesium diet to the extent of 1.4 per cent to growing calves caused reduction in serum calcium level to the extent of 8.6 mg/dl at 9th week of experiment.

The data obtained on serum calcium concentration during the present study is in accordance with the results reported by Packett and Hauschild (1964), James (1968, 1973) and Kallfelz *et al.* (1986). It can be further stated that decreased calcium absorption may be attributed to an increased urine magnesium, perhaps as a result of decreased tubular resorption of calcium in the presence of excess magnesium in the tubular fluid (Kallfelz *et al.*, 1986). The result also tend to suggest that supplementation of ammonium chloride suppressed the effect of high dietary magnesium on serum calcium and maintained its level within the normal range. Supplemental horse gram extract was less effective than ammonium chloride in this regard, however, there is no statistical evidence to support this finding.

5.5.2.2 Phosphorus

Data presented in Table 19, represented by Figure 5 and that summarised in Table 21 with regard to serum inorganic phosphorus showed a.gradual rise in serum inorganic phosphorus concentration during the progress of experiment, the average values (mg/dl) obtained during the course of the experiment being 6.47, 5.69 and 6.08 for the animals maintained on rations A, B and C respectively. Statistical analysis of the data presented in Table 22, indicate a significant ($P<0.01$)

rise in serum inorganic phosphorus in goats maintained on group I when compared to that of groups II and III, while there is no significant difference between the groups II and III and a significant difference ($P<0.01$) between groups I versus II and I versus III indicating that high dietary magnesium enhances the absorption of phosphorus and since there is no significant rise in serum inorganic phosphorus exhibited by the groups II and III when compared to group I may be attributed to the influence of supplemental ammonium chloride or horse gram extract, both interfered the absorption of phosphorus or counteracted the action of high dietary magnesium on phosphorus absorption in goats maintained on rations B and C.

Packett and Hauschild (1964) reported in lambs that calculogenic rations having 0.30 and 0.26 per cent calcium, 0.30 and 0.24 per cent phosphorus and 0.22 and 0.21 per cent magnesium enhance the serum phosphorus level (mg/dl) from 6.83 and 6.61 to 10.16 and 9.34 at 11th week of experiment.

Bushman *et al.* (1965) reported that maximum calculogenic incidence in lambs when fed with 0.37 to 0.57 per cent calcium, 0.55 per cent phosphorus and 0.18 per cent magnesium with a serum phosphorus levels of 8.61 and 9.27 mg/dl increased to the extent of 12.07 and 9.51 mg/dl on raising the

dietary magnesium level to the extent of 0.38 per cent in the ration.

James (1973) reported in goats that a ration identical to the ration used in the present study (ration A) when fed to goats caused an increase in serum phosphorus level (mg/dl) from 6.1 to 7.1 during the course of the experiment.

The results obtained during the course of present investigation are in keeping with the findings of above authors (Packett and Hauschild, 1964; Bushman *et al.*, 1965 and James, 1973) and tend to suggest that high dietary supplementation of magnesium in the ration enhances the serum inorganic phosphorus level, due to increased absorption of phosphorus.

5.5.2.3 Magnesium

Serum magnesium concentrations obtained from the three groups of animals under experimentation have been presented in Table 20, represented by Figure 6 and summarised in Table 21, the average serum magnesium values (mg/dl) obtained during the course of the present investigation for the groups I, II and III being 4.78, 3.70 and 4.22 respectively. Statistical analysis of the data (Table 22) reveal a significant

difference ($P<0.01$) between the three groups, as can be seen from the fact that the group I versus II, I versus III and II versus III, significantly ($P<0.01$) differ from each other with respect to serum magnesium concentration, indicating that supplemental ammonium chloride and supplemental horse gram extract have got profound influence on reducing the serum magnesium concentration in the respective groups, but the effect is more pronounced in ammonium chloride supplemented group (group II) than horse gram extract supplemented group (Group III).

James (1968) reported in goats that a ration containing 0.386 per cent calcium, 0.267 per cent phosphorus and 0.6 per cent magnesium caused a rise in serum magnesium concentration (mg/dl) from 2.3 to 5.4. James (1973) also reported in goats fed a high magnesium calculogenic ration exhibited a rise in serum magnesium level from 3.2 to 5.2 mg/dl.

Chicco *et al*. (1973) reported that lambs fed with ration having 0.14 and 0.42 per cent calcium, 0.12 and 0.36 per cent phosphorus with magnesium level raising from 500 ppm to 5500 ppm exhibited an increase in serum magnesium level from 1.88 to the extent of 3.45 mg/dl.

Kallfelz *et al.* (1986) also demonstrated in calves that elevation of dietary magnesium level cause, elevation in serum magnesium concentration. Petersson *et al.* (1988) reported in calves when fed a ration containing 0.80 per cent calcium, 0.75 per cent phosphorus, when increasing the dietary magnesium level from 0.10 to 0.30 per cent, cause a rise in serum magnesium level from 1.60 to 1.76 mg/dl.

From a perusal of the results obtained, it has been observed that raising the dietary magnesium level cause elevated serum magnesium level which lend further evidence to support the view of James (1968, 1973), Chicco *et al.*, 1973; Kallfelz *et al.* (1986) and Petersson *et al.* (1988).

A rise in serum magnesium level due to feeding of excess magnesium may be attributed to an increased absorption of dietary magnesium. However, supplementation of ammonium chloride evince a significant reduction in serum magnesium concentration, possibly due to decreased magnesium absorption, indirectly due to increased calcium absorption with supplemental ammonium chloride in the present investigation or due to the interaction between these two elements namely, calcium and magnesium. The effect of horse gram extract is less efficient than that of supplemental ammonium chloride in this regard.

5.6 Mineral and nitrogen balance studies

5.6.1 Urine values

5.6.1.1 Calcium

Urine calcium values obtained at monthly intervals during the experimental period from the animals maintained on three groups (group I, II and III) are presented in Table 23 to 26, summarised in Table 39, the initial calcium excretion (g/day) being 0.104, 0.109 and 0.108 respectively, gradually increased during the progress of experiment in all the groups and at the end of the experiment (84th day) the urine calcium excretion recorded as 0.191, 0.128 and 0.151 g/day. Statistical analysis of the data presented in Table 42 disclose that the goats maintained under group I excreted significantly ($P<0.01$) higher urine calcium when compared to groups II and III, as can be seen from a significant difference ($P<0.01$) exist between groups I and II and groups I and III respectively, while no significant difference in urine calcium could be noticed between goats maintained under groups II and III. The data tend to suggest that the decreased utilization of calcium in animals maintained on group I may be due to the influence of high dietary magnesium, but supplemental ammonium chloride in group II and supplemental horse gram extract in group III

have counteracted the influence of dietary magnesium over calcium utilization as was evidenced from the fact that there was no significant difference discernible between the groups II and III and both differs from group I.

The urine calcium concentration (mg/dl) obtained from the three experimental groups before feeding experimental ration were 7.542, 7.334 and 7.956 and on the termination of experiment the concentration of calcium excreted through urine were 12.862, 5.587 and 7.968 respectively in groups I, II and III and are depicted in Figure 7.

James (1968) reported that a ration having 0.386 per cent calcium, 0.267 per cent phosphorus and 0.6 per cent magnesium caused an increase in urine calcium (g/day) from 0.073 to 0.138 and suggested that increased dietary magnesium interfered with the utilization of calcium. In another investigation James (1973) observed that the initial urine calcium concentration in goats ranging from 0.077 to 0.090 g per day had been raised to 0.118 to 0.153 g per day at the experimental period of 112th day, when fed a ration identical to that used during the course of present investigation (ration A).

Kallfelz *et al.* (1986) reported that a ration having 1.2 per cent magnesium when fed to growing calves cause a decrease in serum calcium, might be due to increased serum calcium excretion through urine and concluded that the increase in urine calcium excretion perhaps as a result of decreased renal tubular reabsorption of calcium in the presence of an excess of magnesium in tubular fluid.

Petersson *et al.* (1988) reported that the calcium concentration (mg/dl) in urine were increased from 75.39 to 77.15 when magnesium level in the ration enhanced from 0.3 to 0.6 per cent along with low water intake, but with increased water intake at both levels of magnesium the urine calcium concentration (mg/dl) were 91.26 and 96.04 and concluded that increased supplementation of magnesium enhanced calcium concentration in urine of calves.

The result obtained during the course of present investigation lend further evidence to support the findings of above authors (James, 1968, 1973; Kallfelz *et al.*, 1986 and Petersson *et al.*, 1988). It can also be noted that supplementation of ammonium chloride in animals maintained under group II cause a reduction in calcium concentration (mg/dl) and total excretion of calcium per day, which may be attributed to better utilization of calcium than goats

maintained on group III and even significantly better than goats maintained on group I.

5.6.1.2 Phosphorus

Urine phosphorus values presented in Tables 27 to 30 and that summarised in Table 39 showed an appreciable increase in urine phosphorus level from 0.182 to 0.530, 0.197 to 0.231 and 0.199 to 0.275 g per day for the groups I, II and III respectively indicating that the rise in urine phosphorus excretion is more pronounced in animals maintained on group I. Statistical analysis of the data presented in Table 42 disclose a significant ($P<0.01$) rise in urine phosphorus excretion in group I when compared to that of animals maintained on groups II and III, as is evidenced from a statistical significance ($P<0.01$) between the groups I versus II and I versus III indicating that dietary magnesium interferes with the utilization of phosphorus, however, no significant difference is discernible between the groups II and III but the animals maintained on group II had appreciably lower urine phosphorus excretion than group III. On further scrutiny of the data, it can be seen that supplementation of ammonium chloride along with high magnesium calculogenic ration (ration B) significantly reduced the urine phosphorus excretion but less pronounced in goats maintained on

supplemental horse gram extract (ration C), indicating that both supplemental ammonium chloride and horse gram extract has got influence on phosphorus utilization as is evidenced from a statistically significant decrease in urine phosphorus excretion in groups II and III when compared to group I.

The urine phosphorus concentration (mg/dl) also had a similar trend and the values at the beginning of experiment were 13.197, 13.255 and 14.661, which have been augmented at the end of the experimental period as 35.690, 10.083 and 14.512 for the animals maintained on groups I, II and III as represented by Figure 8.

James (1973) reported in goats that a ration supplemented with dietary magnesium to the extent of 1.102 per cent level caused a significant increase in urine phosphorus and suggested that high dietary magnesium interfered with the utilization of phosphorus thereby increased urinary phosphorus excretion and the result obtained during the course of present investigation lend further evidence to support this view.

5.6.1.3 Magnesium

Urine magnesium values presented in Tables 31 to 34 and that summarised in Table 39, showed an initial magnesium

excretion of 0.220, 0.190 and 0.212 g per day have been increased to the extent of 1.617, 1.580 and 1.590 g per day respectively for animals maintained on groups I, II and III and the statistical analysis of the data presented in Table 42 revealed no significant difference between the groups with regard to magnesium excretion through urine indicating that neither supplementation of ammonium chloride nor horse gram extract has got any significant influence on the utilization of magnesium. However, it can be seen from the Table 39 that an increased level of magnesium in the ration causes an appreciable increase in urine output of this element.

The concentration of magnesium in urine represented by Figure 9 at the beginning and at the end of the experiment being 15.954 to 108.889, 12.785 to 68.966 and 15.619 to 83.905 mg per 100 ml for the animals maintained on groups I, II and III respectively. These results tend to show that supplementation of ammonium chloride contribute to a reduction in urine magnesium concentration (mg/dl) followed by horse gram extract.

Bushman *et al.* (1965) reported in lambs fed with various levels of calcium ranging from 0.37 to 1.27 per cent, phosphorus from 0.25 to 0.55 per cent and on increasing the magnesium level from 0.18 to 0.38 per cent cause an increase

in urine magnesium excretion from 107 mg per day to as high as 210 mg per day.

James (1968) observed that when goats fed 0.6 per cent magnesium, the urine magnesium values vary from 0.73 to 0.138 g per day, and the concentration was increased from 13.4 to 24.9 mg per day. In another investigation James (1973) observed that at 1.102 per cent magnesium in the diet, the urine magnesium increased from 0.308 to 1.60 g per day and the concentration of magnesium increased to the extent of 160 to 204 mg from 35 to 45 mg per 100 ml.

Chicco *et al.* (1973) reported in sheep that feeding a diet having 750 ppm magnesium with 0.18 and 0.78 per cent calcium, the excretion of magnesium in urine was 0.090 and 0.188 g per day which had been increased to the extent of 1.324 and 1.337 g per day respectively when the dietary magnesium has been increased to the extent of 7750 ppm and suggested that increasing the dietary magnesium causes increased excretion of magnesium through urine.

Gentry *et al.* (1978) observed that when calves fed 4 per cent added magnesium, the urine magnesium increased from 64 to 580 ppm while in those calves given 2 per cent added magnesium, the values rose from 56 to 273 ppm.

Petersson *et al.* (1988) also observed in veal calves when fed at different concentration of magnesium (0.1, 0.3 and 0.6 per cent) the urine magnesium excretion were 172.02, 354.93 and 531.38 g per day respectively and the concentration of magnesium (mg/dl) were 3.86, 7.56 and 14.13 respectively.

The result obtained during the present study is in agreement with the above reports (Bushman *et al.*, 1965; James 1968 and 1973; Chicco *et al.*, 1973; Gentry *et al.*, 1978 and Petersson *et al.*, 1988).

5.6.1.4 Nitrogen

Urine nitrogen values gathered during the present investigation are presented in Tables 35 to 38 and that summarised in Table 39, the urine nitrogen excretion (g/day) at 0 day and 84th day of experiment being 6.865 and 11.445, 6.610 and 10.953 and 6.618 and 10.907 from goats maintained on groups I, II and III respectively. The statistical analysis of the data presented in Table 42 revealed no significant variation in nitrogen excretion between the three groups indicating that neither supplementation of ammonium chloride nor horse gram extract have influenced the urine nitrogen excretion.

James (1973) reported in goats fed with high magnesium to the extent of 1.102 per cent recorded a urine nitrogen excretion of 4.990 g per day to 5.813 g per day against control group which excreted 5.534 and 6.430 g per day during the initial (0 day) and final period (112th day) and concluded that supplementation of minerals had not influenced the nitrogen excretion in urine.

The result obtained on urine nitrogen excretion are in accordance with the above finding (James, 1973).

5.6.2 Faecal values

5.6.2.1 Calcium

Data pertaining to faecal calcium values are presented in Tables 23 to 26 and that summarised in Table 40 with their statistical analysis in Table 43, reveal no significant difference between the groups. The faecal calcium excretion recorded (g/day) at the termination of experiment for the animals maintained on three groups I, II and III being 5.115, 4.304 and 4.472 respectively, indicating that neither supplemental ammonium chloride nor supplemental horse gram extract influenced the faecal calcium excretion.

James (1968) reported that goats fed ration having 0.386 per cent calcium, 0.267 per cent phosphorus and 0.237 per cent magnesium recorded faecal calcium excretion of 1.755 g per day but when the dietary magnesium level was increased to 0.6 per cent, the faecal calcium excretion increased to the extent of 1.944 g per day. In another investigation James (1973) reported that a ration having 1.220 per cent calcium, 0.628 per cent phosphorus and 1.102 per cent magnesium when fed to goats, the faecal excretion of calcium was 4.243 g per day, but with 1.102 per cent dietary magnesium the faecal calcium excretion was 4.988 g per day and concluded that supplemental magnesium causes an increased excretion of calcium through faeces consequent on decreased digestibility of calcium.

Chicco *et al.* (1973) reported in lambs that when fed ration having 0.14 and 0.42 per cent calcium, 0.12 and 0.36 per cent phosphorus on increasing the dietary magnesium level from 500 ppm to 5500 ppm cause rise in faecal calcium values from 0.854 and 2.610 g per day to 0.861 to 2.853 g per day.

The results obtained during the present investigation are in keeping with the findings of the above authors (Chicco *et al.*, 1973; James, 1968, 1973).

5.6.2.2 Phosphorus

The values obtained on faecal phosphorus excretion are presented in Tables 27 to 30 and that summarised in Table 40, the faecal phosphorus values for the animals maintained under the three groups viz., group I, II and III at the termination of experiment being recorded as 3.740, 3.530 and 3.613 g per day. Statistical analysis of the data presented in Table 43, reveal no significant difference between the three groups with respect to faecal excretion of phosphorus indicated that neither supplementation of ammonium chloride nor horse gram extract had any significant influence on the digestibility of dietary phosphorus.

James (1968) observed that ration having 0.386 per cent calcium, 0.267 per cent phosphorus and 0.6 per cent magnesium the faecal phosphorus values increased from 0.505 to 0.795 g per day. In another investigation James (1973) observed that goats fed ration identical to the present experimental ration A, the faecal phosphorus values increased from 1.753 to 4.443 g per day and suggested that increasing the dietary magnesium in the ration causes decreased digestibility of phosphorus.

Chicco *et al.* (1973) reported in lambs from three factorial trials involving 2x2x3 treatments with calcium,

phosphorus and magnesium respectively, on increasing the magnesium level in the diet from 500 ppm to 5500 ppm causes increased faecal loss of phosphorus.

So the result obtained on faecal phosphorus excretion is in agreement with those reported by the above authors (James 1968 and 1973; Chicco *et al.*, 1973).

5.6.2.3 Magnesium

The values obtained on faecal magnesium excretion presented in Tables 31 to 34, summarised in Table 40 with their statistical analysis in Table 43 disclose no significant difference between the three groups, the faecal magnesium excretion (g/day) at the end of the feeding trial for the groups I, II and III being recorded as 6.101, 6.503 and 5.973 respectively. Neither supplemental ammonium chloride nor supplemental horse gram extract had any influence on faecal magnesium excretion or digestibility of dietary magnesium in the respective groups (groups II and III).

James (1968) observed in goats when fed with ration having 0.237 per cent magnesium causes a faecal excretion of 0.479 g magnesium per day, had been increased to 0.674 g per day with dietary magnesium levels of 0.6 per cent. In another

investigation James (1973) observed in goats that diet having 1.102 per cent magnesium cause a raise in faecal magnesium excretion to 1.135 g per day at the end of the experiment.

Chicco *et al.* (1973) reported in lambs that faecal magnesium values of 2.010 to 2.853 were obtained by feeding varying levels of dietary magnesium from 500 to 5500 ppm with varying levels of dietary calcium and phosphorus.

The result obtained during the present study is in keeping with the findings of the above authors (James 1968 and 1973; Chicco *et al.*, 1973) who suggested that increasing the dietary magnesium increases the faecal loss of magnesium.

5.6.2.4 Nitrogen

Data presented in Tables 35 to 38 with respect to values on faecal nitrogen excretion from the animals maintained on three experimental rations A, B and C and that summarised in Table 40 with their statistical analysis in Table 43, reveal no significant variation between the experimental groups on faecal nitrogen excretion, the mean values of faecal nitrogen excretion at the termination of experiment (84th day) being recorded as 11.610, 11.997 and 11.665 g per day. Neither addition of ammonium chloride nor horse gram extract had any

appreciable effect on nitrogen digestibility of animals maintained under the respective group.

James (1968, 1973) observed in goats that a dietary magnesium level of either 0.6 per cent or 1.102 per cent had no influence on faecal nitrogen excretion and concluded that supplementation of magnesium in the ration did not have any influence on digestibility of nitrogen.

The result obtained during the present study is in acceptance with the above reports (James, 1968 and 1973).

5.6.3 Percentage retention

5.6.3.1 Calcium

The data gathered on per cent retention of calcium presented in Tables 23 to 26 represented by Figure 10 and that summarised in Table 41 with their statistical analysis in Table 44, reveal a significant rise in calcium balance during the progress of experiment in group II when compared to that of groups I and III, the values of per cent retention of calcium gathered from the three experimental groups I, II and III at the beginning (0 day) and at the end (84th day) of experiment being 62.05 and 59.85, 62.71 and 66.55 and 61.04 and 62.65 per cent respectively, showing that supplemental

ammonium chloride enhanced the retention of calcium as evidenced from significant difference between the groups II versus I and groups II versus III and supplemental horse gram extract has not shown any significant influence on per cent retention of calcium as can be seen from a non significant difference between the groups I and III.

James (1973) observed in goats that a ration having 1.220 per cent calcium, 0.628 per cent phosphorus with 0.6 per cent magnesium there was increased retention of calcium at the termination of experiment (112th day) from 52.5 to 61.6 per cent, but on increasing the dietary magnesium level to the extent of 1.102 per cent, there was reduction in per cent retention of calcium from 60.2 to 52.4 per cent.

So the result obtained with regard to per cent retention of calcium from group I fed calculogenic ration (ration A) is in keeping with the finding of James (1973) who suggested that high dietary magnesium at 1.102 per cent level interfered the per cent retention of calcium.

5.6.3.2 Phosphorus

Data gathered on phosphorus retention are presented in Tables 27 to 30, represented by Figure 11 and that summarised

in Table 41 with their statistical analysis in Table 44 reveal a significantly better phosphorus balance during the progress of experiment in group II, when compared to that of groups I and III, the values on per cent retention of phosphorus obtained from the three experimental groups (groups I, II and III) at the beginning (0 day) and at the termination (84th day) of the experiment being 46.64 and 35.24, 45.28 and 43.24 and 47.48 and 37.72 per cent respectively, indicating that supplemental ammonium chloride enhanced the retention of phosphorus as evidenced from a significant difference between the groups II versus I and II versus III. Supplemental horse gram extract also shown a tendency to enhance the retention of phosphorus, however, the effect is not being statistically significant.

James (1968) observed in goats that a ration having 0.386 per cent calcium, 0.267 per cent phosphorus and 0.6 per cent magnesium had a reduction in phosphorus balance from 30.2 to 25.5 per cent at the end of the experiment. In another investigation James (1973), observed a significant reduction in per cent retention of phosphorus in goats from 56.4 to 30.2 per cent and concluded that high dietary magnesium causes reduction in per cent retention of phosphorus in goats.

The result obtained during the present investigation with respect to a reduction in per cent retention of phosphorus in group I may be attributed to the high dietary magnesium which influenced the per cent retention of phosphorus and this result lends further evidence to support the view of James (1968 and 1973).

5.6.3.3 Magnesium

Per cent retention values of magnesium obtained during the course of present investigation are shown in Tables 31 to 34, represented by Figure 12 and consolidated in Table 41, the values recorded for the three groups at the beginning and at the end of the experiment being 32.20 and 42.49, 31.86 and 40.01 and 34 and 41.80 per cent for the groups I, II and III respectively. Statistical analysis of the data presented in Table 44 disclose no significant difference between the groups with regard to per cent retention of magnesium. On scrutiny of the data it can be observed that, in all three groups the per cent retention values have been increased towards the termination of the experimental period, but the increase was more pronounced in group I supplemented with high dietary magnesium but less pronounced in groups III and II in that descending order.

James (1968) reported in goats that, with 0.6 per cent magnesium in a ration, the per cent retention of the element increased from 21.5 to 73.9 per cent at the end of the experiment. James (1973) observed in goats fed with a ration identical to the present experimental ration A, the magnesium retention increased from 62.4 to 72.6 per cent and concluded that increasing the level of dietary magnesium causes increase in per cent retention of the element.

The result obtained on per cent retention of magnesium is in keeping with the previous findings (James 1968 and 1973).

5.6.3.4 Nitrogen

Data on per cent retention of nitrogen presented in Tables 35 to 38, represented by Figure 13 and consolidated in Table 41 with their statistical analysis in Table 44 indicate, no significant difference among the three groups with regard to per cent retention of nitrogen, the values recorded for the three groups at the beginning and at the end of the experiment being 32.6 and 41.30, 34.35 and 43.50 and 31.43 and 40.65 per cent for the groups I, II and III respectively. It can be seen further from the data that in all the three groups there is enhancement of per cent retention of nitrogen towards the termination of experiment, the extent of per cent nitrogen

retention is better in group II, I and III in that descending order.

James (1973) observed in goats when fed with ration identical to the present experimental ration did not have any influence on nitrogen retention.

The result obtained during the present investigation is in keeping with the above finding (James, 1973). The result also tends to show an increased retention of nitrogen due to supplemental ammonium chloride but the effect is not statistically significant.

Examination of urine

Microscopical examination of urine samples collected from group I fed on calculogenic ration A showed severe crystalluria when compared to that of animals maintained on group III fed on calculogenic ration A supplemented with horse gram extract while group II fed on calculogenic ration A supplemented with ammonium chloride did not exhibit any crystalluria during the progress of experiment. These results indicate that calculogenic ration containing 1.194 per cent calcium, 0.578 per cent phosphorus and 1.202 per cent magnesium produce severe cystalluria. Supplementation of

horse gram extract with the calculogenic ration cause reduction in intensity of crystalluria whereas supplemental ammonium chloride prevented the crystalluria. James (1968 and 1973) reported that a ration having high magnesium either at 0.6 per cent or 1.102 per cent caused crystalluria in goat as was observed during the course of the present investigation.

Further on microscopical examination of the crystals collected from urine samples obtained from group I and III reveal that the crystals were having typical prismatic "coffin lid" shape which is a characteristic shape of struvite crystals. Chemical analysis (spot test by Winer, 1959) of the crystal confirmed the presence of ammonium, magnesium and phosphate.

Severe crystalluria and consequent development of urinary calculi exhibited by group I may be explained in such a way that when high magnesium were fed, the urine concentration of magnesium and phosphorus will be increased and also the urinary urea which is easily decomposed to ammonium ions resulting in high levels of magnesium, phosphate and ammonium ions in the urine - a condition optimal for precipitation of highly insoluble magnesium ammonium phosphate (struvite). Similar mechanism for the formation of struvite calculi was also suggested by McIntosh (1978). Katheleen Lonsdale (1968)

indicated that the passage of sandy urine, however, should be taken as a danger signal because of epitaxial deposit.

The urine samples obtained from group I had an average pH of 8.4 (alkaline) which was also reported by Bushman *et al.* (1965) in lambs and James (1968 and 1973) in goats and they concluded that supplemental calcium, phosphorus and magnesium did not exert any influence on the pH of urine. Larson (1996) indicated that normal urine pH of ruminants ranging between 7 to 9.5 favours the precipitation of triple phosphate crystals. So the result obtained during the present study is in keeping with the previous reports (Bushman *et al.*, 1965; James, 1968 and 1973 and Larson, 1996).

Goats maintained on group II fed on supplemental ammonium chloride excreted significantly low concentration of urine calcium, phosphorus and magnesium than the animals maintained on groups I and III, which reduced the supersaturation and crystallisation by avoiding precipitation of these crystalloids as evidenced by absence of crystalluria in group II. The average pH of urine collected from group II had an appreciable reduction in pH from 8.4 to 8.0.

Several authors observed that supplemental ammonium chloride cause a significant change in urine pH from alkaline

to acidic, which prevented the crystal growth and calculi formation (Crookshank *et al.*, 1960; Bushman *et al.*, 1967, 1968; Yano and Kawashima, 1977 and Stewart *et al.*, 1991). Such a significant reduction in urine pH from alkaline towards acidic was not observed during the present study, however, it seems that goats fed ration containing supplemental ammonium chloride had a slight reduction in urine pH. Such a reduction of urine pH may be attributed to the excess chloride ions supplied by the supplemental ammonium chloride as was observed by Jit Singh and Kuldip Singh (1990).

It was also noted that goats maintained on group II, had an appreciable increase in urine volume (1860.93 ml per day) than the animal maintained under group I (1412.9 ml per day) which prevent supersaturation of urine by the crystalloids and consequent crystal growth.

Goats maintained under group III fed on calculogenic ration supplemented with horse gram extract had decreased intensity of crystalluria and excreted urine with an average pH of 8.3.

Microscopical examination supported by chemical analysis, revealed that the crystals are similar in chemical composition (struvite) to that obtained in group I. The possible

reduction in the intensity of crystalluria in group III when compared to that of group I, may be due to decreased excretion of phosphorus and magnesium than that excreted by the animals under group I, but the concentration of these elements were higher than that obtained from group II. Also the average urine volume excreted by group III was appreciably higher (1774.74 ml per day) than that of group I (1412.90) and comparable to group II (1866.93), thereby decreases the concentration of crystalloids and supersaturation of urine that prevented the crystal formation.

Autopsy and histopathological observation

Group I

On post mortem examination the kidney collected from animals in group I showed moderate enlargement with white patches and focal haemorrhages over the cortex. Pelvis of the kidney had numerous visible sand like rudimentory calculi. Bladder had thickened mucosal layer with focal haemorrhages and erosions. No calculus material was recovered from the ureter, bladder or urethra, but urethra had hyperemic changes near to neck of the bladder. These findings confirm the possible calculogenic effect of the experimental ration A.

Similar findings were reported by James (1968 and 1973) in goats and in addition, also observed postmortem lesions such as distention of perirenal tissues with dark yellowish red urine due to obstruction by visible calculi, dark yellowish red urine in the bladder with distention due to obstruction caused by visible calculi and calculus material recovered from the urethra, when the animals were maintained on the calculogenic ration identical to that used for the present investigation. Such findings were not fully observed in the present investigation perhaps due to shorter experimental period (84 to 100 days) when compared to that of the above report in which the goats were maintained on a calculogenic ration for a period of 126 days (James, 1973).

Histopathological examination of kidneys collected from animals (group I) maintained on ration A showed severe destruction of tubular epithelium, dilated tubules with proteinacious casts and microcalculi of varying stages of development, dilated blood vessels with infiltration of inflammatory cells in the intestitial area. These findings were also observed by James (1968 and 1973). Bladder mucosal cells were engorged and sloughed out in some places. Both ureter and urethra did not show any appreciable change since there was no obstructive urolithiasis in the ureter or in the urethra during the present investigation.

Autopsy and histopathological observations confirm the calculogenic effect of ration A (basal calculogenic ration) to goats.

Group II

On postmortem examination of urinary organs collected from goats (group II) fed on Ration B supplemented with ammonium chloride had slight enlargement of the kidney. No visible calculi could be located in any part of the urinary tract or any other gross lesions in the urinary organs of goats.

On histopathological examination of the kidneys, tubular epithelium had hyalinization with some tubules exhibiting pink stained proteinacious casts and having focal areas of inflammation with infiltration of inflammatory cells and proliferation of fibrous tissues in between the tubules. No microcalculi could be detected in the tubules nor any appreciable change in the bladder or urethra.

These observations tend to suggest that ammonium chloride exerts a beneficial effect on the prevention of calculi formation in goats when fed along with high magnesium calculogenic ration.

Group III

On postmortem examination, kidney had slight enlargement with focal areas of haemorrhages and had few greyish white patches over the cortex. The pelvis of the kidney had few rudimentary sand like calculus material. Bladder had focal areas of haemorrahges on the thickened mucosal surface. No visible calculus material could be recovered from bladder, ureter or urethra, but urethra had slight hyperemic change near the neck of the bladder.

On histopathological examination of the kidney, lesions are comparable to that observed in goats fed on ration A (group I). Ureter, and urethra did not show any appreciable histopathological changes. Bladder had thickening of mucosa and infiltration of inflammatory cells.

The gross and histological lesions observed are comparable to group I wherein, the kidney had traces of calculus material and few microcalculi, inclined to suggest that horse gram extract is not effective in preventing the calculi formation completely.

From a critical evaluation of the gross and histopathological changes observed in animals maintained on

rations A, B and C respectively, it can be concluded that the animals maintained on ration A had higher degree of kidney changes. Animals receiving ration B had not shown much histological changes and animals maintained on ration C had varying degree of kidney changes.

On recapitulating the results on biochemical changes observed on maintaining goats on different dietary treatments, it can be surmised that a ration is prone to be calculogenic, if it induce high serum magnesium and phosphorus, and low serum calcium, more excretion of phosphorus and magnesium through urine with less retention of calcium and phosphorus and higher retention of magnesium, consequently found visible lesions and histopathological changes in the urinary organs as that observed during the course of present investigation (group I).

The most obvious explanation of the effect of the chloride ion is on the solubility of the calculi forming solutes (Petersson *et al.*, 1988, Jit Singh and Kuldip Singh, 1990). In a solution such as urine which is both concentrated and complex in composition, the interaction between the ions and their solubilities are not readily predictable and struvite or magnesium ammonium phosphate is a highly insoluble

salt above a pH of about 7 (Larson, 1996), whereas magnesium chloride is highly soluble, the balancing of these two anions excreted is important in establishing the condition least favourable for the formation of calculi. If a ration is designed to reduce the load of phosphate excreted, an increase in chloride excretion would have the greatest probability of minimising calculus formation as occurred in animals maintained on ration B (group II).

Hardly any information is available regarding the possible beneficial action of horse gram extract in the prevention of urolithiasis in animals except that reported by Das (1956). However, the present study indicate that supplemental horse gram extract had a tendency to reduce urine phosphorus concentration and increased the volume of urine, which may not be sufficient to prevent the calculi formation completely as evidenced by autopsy and histopathological findings. Perhaps, the supplemental horse gram extract could have been exerted its beneficial effect if the experimental period is extended few more days.

From a critical evaluation of the overall results obtained during the course of the present investigation on the efficacy of ammonium chloride and horse gram extract in the

amelioration of urinary calculi in goats, it can be reasonably concluded that incorporating ammonium chloride to the extent of one per cent in the ration of goats certainly play a positive role in the amelioration of urinary calculi but the action of horse gram extract is doubtful in this regard.

Summary

6. SUMMARY

An investigation spread over a period of 84 days was carried out using Malabari goats to assess the efficacy of ammonium chloride and horse gram (Dolichos biflorus) extract on the amelioration of urolithiasis in goats. Eighteen adult male Malabari goats of 9-12 months old, with an average body weight of 19.4 kg were divided randomly into three groups (I, II and III) of six animals each as uniformly as possible with regard to age and body weight. Basal calculogenic ration A was constituted by using conventional feed ingredients supplemented with calcium, phosphorus and magnesium to an estimated level of 1.194, 0.578 and 1.202 per cent respectively. Experimental ration B was prepared by supplementing ammonium chloride to the extent of one per cent level to basal concentrate ration A. Experimental ration C was formed by feeding horse gram extract to the extent of one litre per animal per day along with basal calculogenic ration A. Groups I, II and III were fed with rations A, B and C respectively.

All the animals were fed individually at maintenance level with weighed quantities of concentrate and roughage at the ratio of 4:1 along with wholesome water provided

ad libitum throughout the experimental period. Daily feed intake and weekly body weight gain were recorded throughout the experimental period. Blood samples were collected at monthly intervals for haematological studies. Digestion cum balance trials (five days collection period) were conducted before starting of experiment and at monthly intervals till the termination of feeding trial. At the end of the experiment all the experimental animals were slaughtered and the urinary organs were collected and subjected to detailed postmortem and histopathological studies.

The criteria used for evaluation of the rations (rations A, B and C) were body weight daily gain, dry matter consumption, feed efficiency, protein efficiency, haematological parameters, data gathered from digestion cum balance trials, examination of urine, atopsy and histopathological examination.

Goats maintained on group I, II and III fed on basal calculogenic ration (ration A), calculogenic ration supplemented with ammonium chloride (ration B) and calculogenic ration supplemented with horse gram extract (ration C) recorded an average cumulative weight gain and average daily gain during the experimental period as 5.20, 5.29 and 5.17 kg and 61.90, 62.98 and 61.55 g per day

respectively, the values being not statistically significant between the groups indicating that neither supplemental ammonium chloride nor horse gram extract had any significant influence on the body weight gain of goats, however, group II had a better weight gain than the animals maintained under groups I and III.

The dry matter consumption recorded for the three groups I, II and III were 928.57, 917.98 and 901.38 g per day respectively, had not shown any statistical significance between the three groups. Feed efficiency and protein efficiency values for the animals maintained on rations A, B and C being recorded as 15.02 and 2.69, 14.58 and 2.60 and 14.69 and 2.62 respectively, did not exhibit any significant variation among the three groups, indicated that neither supplementation of ammonium chloride nor horse gram extract had any significant influence on the dry matter intake, feed efficiency and protein efficiency but group II fed supplemental ammonium chloride had a tendency towards better feed efficiency and protein efficiency than groups I and III.

Haematological parameters such as TEC, TLC, haemoglobin and total protein obtained from three groups at monthly intervals, during the experimental period and no significant difference between the groups indicated that neither

supplemental ammonium chloride nor horse gram extract had any significant influence on these haematological parameters. The values recorded were within the normal range throughout the course of experiment showing that the animals were in normal nutritional status.

The average serum calcium values (mg/dl) recorded for three groups being 8.67, 9.29 and 8.99 respectively for the groups I, II and III. There was significant ($P<0.01$) reduction in serum calcium concentration in group I receiving ration A when compared to that of groups II and III receiving rations B and C, whereas no significant difference exhibited between the groups II and III. The results indicated that high dietary magnesium interfered with the absorption of calcium but supplementation of ammonium chloride and horse gram extract have significantly counteracted the influence of high dietary magnesium over calcium absorption in that descending order, and maintained the serum calcium concentration within the normal range.

The average serum phosphorus values (mg/dl) obtained during the course of experiment being 6.47, 5.69 and 6.08 for the animals maintained on groups I, II and III, indicated that high dietary magnesium enhances the absorption of dietaryphosphorus, but supplemental ammonium chloride or horse

gram extract interfered with the absorption of phosphorus or counteracted the action of high dietary magnesium on phosphorus absorption and maintained the serum inorganic phosphorus concentration within the normal range.

Average serum magnesium concentration (mg/dl) obtained from the three groups being 4.78, 3.70 and 4.22 respectively for the groups I, II and III, exhibited significant ($P<0.01$) difference among the three groups, indicated that increasing the dietary magnesium caused elevated serum magnesium due to increased magnesium absorption, whereas supplemental ammonium chloride and horse gram extract had profound influence on reducing the serum magnesium concentration in the respective groups, but the effect was more pronounced by supplemental ammonium chloride than horse gram extract.

Urine calcium values recorded for three groups I, II and III at the beginning and at the end of the experimental period were 0.104 and 0.191, 0.109 and 0.120 and 0.108 and 0.151 g/day respectively. Statistical analysis disclosed that goats in group I excreted significantly high ($P<0.01$) urine calcium compared to groups II and III, while no significant difference between groups II and III, indicated that high dietary magnesium interfered with the utilization of calcium whereas supplemental ammonium chloride and horse gram extract had

counteracted the influence of high dietary magnesium over calcium utilization.

Urine phosphorus values (g/day) obtained from three experimental groups I, II and III at the beginning and at the end of the experiment being 0.182 and 0.530, 0.197 and 0.230 and 0.199 and 0.275 g per day respectively. Statistical analysis disclosed that there was significant ($P<0.01$) rise in urine phosphorus excretion in group I when compared to that of animals maintained on groups II and III, indicated that high dietary magnesium interferes with the utilization of phosphorus, however, no significant difference was discernible between the groups II and III, which indicated that, supplemental ammonium chloride significantly reduced the urine phosphorus excretion and increased its utilization whereas the effect is less pronounced in goats maintained on supplemental horse gram extract.

Data on urine magnesium (g/day) gathered at the beginning and at the end of the experiment being 0.220 and 1.617, 0.190 and 1.580 and 0.212 and 1.159 g per day for the animals maintained on groups I, II and III respectively, indicated that no significant variation between the groups with regard to magnesium excretion through urine. These results indicated that high dietary magnesium cause increased excretion of this

element through urine. Neither supplementation of ammonium chloride nor horse gram extract had any significant influence on the utilization of magnesium.

Urinary nitrogen excretion (g/day) at the beginning and at the termination of the experiment being 6.904 and 11.445, 6.319 and 10.953 and 6.854 and 10.947 from goats maintained on groups I, II and III respectively, had not shown any significant difference between the three groups indicated that neither supplementation of ammonium chloride nor horse gram extract had any significant influence on nitrogen utilization.

Data on faecal calcium, phosphorus, magnesium and nitrogen obtained from the three groups I, II and III at the termination of the experiment being 5.112, 4.304 and 4.472 g per day for calcium, 3.70, 3.530 and 3.613 g per day for phosphorus, 6.101, 6.503 and 5.973 g per day for magnesium and 11.610, 11.997 and 11.665 g per day for nitrogen respectively. Statistical analysis of the data revealed no significant difference between the three groups with regard to faecal excretion of calcium, phosphorus, magnesium and nitrogen and had no influence on dietary nitrogen. Neither supplementation of ammonium chloride nor horse gram extract when fed along with high magnesium calculogenic ration had any significant influence on digestibility of dietary calcium, phosphorus,

magnesium and nitrogen as evidenced by non significant difference between the three groups in this regard.

Average per cent calcium retention of goats maintained on groups I, II and III at the beginning and at the end of the experiment being 62.05 and 59.85, 62.71 and 66.55 and 61.04 and 62.65 per cent respectively, indicated that high dietary magnesium in the diet interfered with the per cent retention of calcium. There was significant ($P<0.01$) rise in calcium balance during the progress of experiment in group II when compared to that of groups I and III and no significant difference among groups I and III, indicated that supplemental ammonium chloride enhance the retention of calcium, whereas supplemental horse gram extract had not shown any significant influence on per cent retention of calcium, but had a trend towards better per cent retention of calcium.

Data gathered on per cent retention of phosphorus from three groups I, II and III at the beginning and at the end of the experiment being 46.64 and 35.24, 45.28 and 43.24 and 47.48 and 37.72 per cent respectively, revealed a significantly ($P<0.05$) higher phosphorus balance during the progress of experiment in group II when compared to that of groups I and III, indicated that high dietary magnesium interfered with the per cent retention of phosphorus, while

supplemental ammonium chloride enhanced the retention of phosphorus. Supplemental horse gram extract shown only a tendency to enhance the retention of phosphorus.

Data on per cent retention of magnesium obtained from the three groups I, II and III during the beginning and at the end of the experiment being 32.20 and 42.49, 31.85 and 40.01 and 34 and 41.80 respectively, revealed no significant difference between the groups, indicated that higher the dietary magnesium, higher will be the per cent retention of magnesium. Neither supplementation of ammonium chloride nor horse gram extract had any significant influence on per cent retention of magnesium when fed along with high magnesium calculogenic ration. Per cent retention of magnesium values in all three groups have been increased towards the termination of the experimental period, but the increase in magnesium retention was more pronounced in groups I, III and II in that descending order.

Per cent retention of nitrogen recorded for the three groups during the beginning and at the termination of the experiment being 32.6 and 41.30, 34.35 and 43.50 and 31.43 and 40.65 per cent for the groups I, II and III respectively, had no significant difference between the three groups with regard to per cent retention of nitrogen indicated that high dietary

magnesium alone or in combination with either supplemental ammonium chloride or horse gram extract had any significant influence on per cent retention of nitrogen. The per cent nitrogen retention was increased in all the three groups, the extent of per cent nitrogen retention is better in group II, I and III in that descending order.

Microscopical examination of urine for the presence of crystalluria, revealed that crystalluria was absent in group II where as, group III had comparatively less intensity of crystalluria than group I, indicated that supplemental ammonium chloride prevented crystalluria but not by supplemental horse gram extract. The urine pH recorded during the experimental period for the groups I, II and III being 8.4, 8.0 and 8.3 respectively indicated that both the dietary treatments had not exerted any profound influence on urine pH. The average urine volume recorded for the three groups being 1412.9, 1866.93 and 1774.74 ml per day respectively for the groups I, II and III indicated the possible diuretic effect of both the dietary treatments (ration A and B) in groups II and III.

On postmortem and histopathological examination, group I exhibited severe gross and histopathological tissue changes in the kidney and bladder and also found to have numerous gross

and microcalculi in the kidney, which confirmed the calculogenic effect of the ration A. Goats (group II) fed ration B had not shown any gross lesions, had only mild histopathological changes in the kidney and bladder and had not found to contain any gross and microcalculi in the kidney, which indicated the possible beneficial effect of ammonium chloride in the prevention of urinary calculi, whereas goats (group III) fed ration C were found to contain less number of gross and microcalculi in the kidney along with gross and histopathological changes comparable to group I, which indicated that horse gram extract is not effective in the prevention of urinary calculi completely, but had a tendency to reduce the incidence of urinary calculi when supplemented along with high magnesium calculogenic ration.

From an overall assessment of the data gathered during the course of present investigation, it can be reasonably concluded that a ration having 1.194 per cent calcium, 0.578 per cent phosphorus and 1.202 per cent magnesium was found to be calculogenic for goats. Supplementation of ammonium chloride along with the high magnesium calculogenic ration at the rate of one per cent level prevented the calculi formation in goats while horse gram extract had not prevented the calculi formation completely but had a tendency to reduce the occurrence of calculi.

References

REFERENCES

*Ahmed, A.S., Amer, H.A. and Ibrahim, I.M. (1990). Influence of dietary mineral imbalance on the incidence of urolithiasis in Egyptian calves. *Vet. Bull.* **43**(1): 73-77.

AOAC (1990). Official methods of analysis. 15th edition. Association of Official Analytical Chemists, Washington, DC.

Archana, P. and Singla, S.K. (1994). Anticalcifying properties of *Dolichos biflorus* (Horse gram) seed. *Indian J. Exp. Bio.* **32**(12): 889-891.

Ashturkar, R.W. (1992). Urolithiasis and left displacement of abomasum in bullock. *Indian Vet. J.* **69**(4): 363.

Ashturkar, R.W. (1994). Urolithiasis in bullocks - a review of 23 cases. *Indian Vet. J.* **71**(5): 489-492.

Bailey, C.B. (1976). Relation of water turn over to formation of silicious calculi. *Can. J. Anim. Sci.* **56**(3): 745-751.

Bailey, C.B., Bezeau, L.M. and Lawson, J.E. (1963). Silica urolithiasis in beef cattle. 5. Effect of controlling urine pH on the incidence and composition of urinary calculi in calves. *Can. J. Anim. Sci.* **43**(1): 150-155.

Bellenger, C.R. Rutar, A.J., Ilkiw, J.E. and Salamon, S. (1981). Urolithiasis in goats. *Aust. Vet. J.* **57**(1): 56.

Bhaskar Singh, K., Venkatasabbaiah, S., Nishat Muklis and Subba Rao, I.V. (1985). Chemical composition of buffalo uroliths and some observations of their occurrence in Andra Pradesh. *Indian Vet. J.* **62**(11): 997-999.

Blood, D.C. and Radostits, O.M. (1989). Veterinary Medcine, VII edition. English Language Book Society (ELBS).

Bushman, D.H., Emerick, R.J. and Embry, L.B. (1965). Experimentally induced ovine phosphatic urolithiasis: Relationships involving dietary calcium, phosphorus and magnesium. *J. Nutr.* **87**(1): 499-503.

Bushman, D.H., Embry, L.B. and Emerick, R.J. (1967). Efficacy of various chlorides and calcium carbonate in the prevention of urinary calculi. *J. Anim. Sci.* **26**(4): 1199-1204.

Bushman, D.H., Emerick, R.J. and Embry, L.B. (1968). Effect of various chlorides and calcium carbonate on calcium, phosphorus, sodium and chloride balance and their relationship on urinary calculi in lambs. *J. Anim. Sci.* **27**(2): 490-496.

Chicco, C.F., Ammerman, C.B., Feaster, J.P. and Dunavant, B.G. (1973). Nutritional interrelationships of dietary calcium, phosphorus and magnesium in sheep. *J. Anim. Sci.* **36**(5): 986-993.

Cotterreau, P., Froget, J., Magot, A., Jaillardon, C. and Monard, G.A. (1966). A case of urinary lithiasis in sheep. *Nutr. Abstr. Rev.* **36**(2): 909.

Crookshank, H.R. (1970). Effect of ammonium salts on the production of ovine urinary calculi. *J. Anim. Sci.* **30**(4): 1002-1004.

Crookshank, H.R., Keating, F.E., Burnett, E., Jones, J.H. and Davis, R.E. (1960). Effect of chemical and enzymatic agents on the formation of urinary calculi in fattening steers. *J. Anim. Sci.* **19**(2): 595-600.

Crookshank, H.R., Packett, L.V.(Jr). and Kunkel, H.O. (1965). Ovine urinary calculi and pelleted rations. *J. Anim. Sci.* **24**(3): 638-642.

Crookshank, H.R. and Robbins, J.D.A. (1962). A reciprocal relationship between the urinary excretion of magnesium and phosphorus in wether lambs. *Nature.* **196**: 1343.

Crookshank, H.R., Robbins, J.D. and Kunkel, H.O. (1967). Relationship of dietary mineral intake to serum mineral level and the incidence of urinary calculi in lambs. *J. Anim. Sci.* **26**(5): 1179-1185.

Cuddeford, D. (1987). Role of magnesium in the aetiology of ovine urolithiasis in fattening store lambs and intensively fattened lambs. *Vet. Rec.* **121**(9): 194-197.

Das, R.C. (1956). Urethral calculi in a bullock and its treatment with the decoction of *Dolichos biflorus* (Horse gram). *Indian Vet. J.* **32**(5): 369-370.

Danowski, T.S. (1962). Clinical Endocrinology, Vol.III. The Williams and Wilkins Company, U.S.A.

Deepa Ananth (1998). Nutritive evaluation of complete ration for growth in kids. M.V.Sc. thesis. Kerala Agricultural University, Mannuthy.

Dutt, B., Majumdar, B.N. and Kehar, N.D. (1959). Vitamin A deficiency and urinary calculi in goats. *Br. Vet. J.* **115**(1): 63-65.

Divers, T.J., Reef, V.B. and Roby, K.A. (1989). Nephrolithiasis resulting in intermittent urethral obstruction in cow. *Cornell Vet.* **79**(2): 143-149.

*Elam, C.J., Ham, W. and Schneider, B.E. (1958). Influence of dietary sodium chloride on incidence of urinary calculi in sheep. *Nutr. Abstr. Rev.* **28**(1): 672.

Emerick, R.J. and Embry, L.B. (1963). Calcium, phosphorus levels to the development of phosphate urinary calculi in sheep. *J. Anim. Sci.* **22**(2): 510-513.

Emerick, R.J., King, H.R. and Embry, H.R. (1972). Influence of dietary potassium and transaconitic acid on mineral metabolism related to ovine phosphatic urolithiasis. *J. Anim. Sci.* **35**(4): 901-907.

Fluss, D.G. (1981). Urolithiasis in goats. *Vet. Rec.* **108**(26): 568.

Gangadevi, P. (1981) Optimum level of protein in concentrate mixture of kids for growth. M.V.Sc. thesis submitted to Faculty of Veterinary and Animal Sciences, Kerala Agricultural University, Mannuthy, Kerala.

Gentry, R.P., Miller, W.J., Pugh, D.G., Neathery, M.W. and Bynum, J.B. (1978). Effects of feeding high magnesiu to young dairy calves. *J. Dairy Sci.* **61**(12): 1750-1754.

Gerald, B. Huntington and Emerick, R.J. (1984). Oxalate urinary calculi in beef steers. *Am. J. Vet. Res.* **45**(1): 180-182.

Godwin, I.R. and Williams, V.J. (1982). Urinary calculi formation in sheep on high wheat grain diet. *Aust. J. Agri. Res.* **33**(5): 843-855.

Harnam Singh and Sadu, S. (1995). Peritoneal lavage as an adjunct therapeutic measure of uremia in bovine. *Indian Vet. J.* **72**(11): 1174-1176.

Hawkins, W.W. (1965). Experimental production and control of urolithiasis. *J. Am. Vet. Med. Assoc.* **147**(12): 1321-1323.

Hoar, D.W., Emerick, R.J. and Embry, L.B. (1970a). Potassium, phosphorus and calcium interrelationship influencing feedlot performance and phosphatic urolithiasis in lambs. *J. Anim. Sci.* **30**(2): 597-600.

Hoar, D.W., Emerick, R.J. and Embry, L.B. (1970b). Influence of dietary calcium source, phosphorus level and acid forming effect of the diet on feedlot performance and urinary calculi formation in lambs. *J. Anim. Sci.* **31**(1): 118.

*Hosseinion, M., Naghshineh, R., Nadalian, M. and Chamma, M. (1976). A case of urinary calculi in a flock of sheep. *Nutr. Abstr. Rev.* **46**(10): 589.

James, C.S. (1968). Enquiry into the etiological factors involved in caprine urolithiasis with special reference to minerals. M.V.Sc. thesis submitted to the University of Kerala.

James, C.S. (1973). An enquiry into the etiological factors involved in caprine urolithiasis with special reference to minerals. Ph.D. theisis submitted to Faculty of Veterinary and Animal Sciences, Kerala Agricultural University, Kerala.

James, C.S. (1978). Studies on goat nutrition. IV. Comparative evaluation of conventional and unconventional feed for evolving cheap and economic ration for goats. *Kerala J. Vet. Sci.* **9**(2): 206-214.

James, C.S. and Mukundan, G. (1978). Suitability of Jack tree leaves as a fodder for stall fed goats. *Indian Vet. J.* **55**(9): 716-721.

Jayakumar, R.M. (1991). Urolithiasis in buck. *J. Vet. Anim. Sci.* **22**(1): 164.

Jethi, R.K., Bindia Duggal, Sahota, R.S., Mridula Gupta and Sofat, I.B. (1983). Effect of the aquous extract of an Ayurvedic compound preparation on mineralization and demineralization reaction. *Indian J. Med. Res.* **78**(9): 422-425.

Jit Singh and Kuldip Singh (1990). Obstructive urolithiasis and uraemia in cattle and buffaloes - a review. *Indian J. Vet. Surg.* **11**(2): 1-20.

Jones, J.O. and Dawson, P. (1976). A urological syndrome in young lambs. *Vet. Rec.* **99**(17): 337-338.

Joshi, P.K., Patil, D.B., Sukumaran, K., Prasania, R.R. and Patel, M.R. (1988a). Effect of Cystone on urine crystallization in bullocks. *Indian J. Vet. Surg.* **9**(1): 18-20.

Joshi, P.K., Patil, D.B., Sukumaran, K., Prasania, R.R. and Patel, M.R. (1988b). A note on the effect of administration of Cystone on pH and electrolyte level in bullocks. *Indian J. Vet. Surg.* **9**(2): 168-170.

Jubb, K.V.F., Kennedy, P.C. and Palmer, N.C. (1991). Pathology of Domestic Animals. (4th edition). Academic Press, Inc. California.

Kallfelz, F.A., Ahmed, A.S., Wallace, R.J., Sasangka, B.H. and Warner, R.G. (1986). Dietary magnesium and urolithiasis in growing calves. *Cornell Vet.* **77**(1): 33-45.

Kathleen Lonsdale (1968). Human stone. Science, **159** (15th MAR): 1199-1207.

Khanna, R.N.S. and Puranchand (1993). A case of hydronephrosis in ovine kidney. *Haryana Agricultural University Journal of Research.* **23**(4): 211-214.

*Koutinas, A., Rallis, T., Psuhas, V. (1989). An outbreak of phosphatic urolithiasis in fattening lambs. *Vet. Bull.* **59**(1): 415.

Kumar, A., Nigam, J.M., Peshin, P.K., Singh, S. and Jani, B.M. (1991). Metabolic changes in blood following obstructive urolithiasis in surviving bullocks. *Indian J. Anim. Sci.* **61**(4): 371-381.

Larson, B.L. (1996). Identifying, treating and preventing bovine urolithiasis. *Vet. Med.* **91**(14):366-377.

Linklater, K.A. and Angus, K.W. (1979). Spontaneous renal disease resembling hyperoxaluria in young lambs. *Vet. Rec.* **104**(19): 429-431.

*Marsh, H. (1961). Urethral occlusion in lambs on feed containing stilbestrol. (Cited in Blood, D.C. and Radostits, O.M. (1989). Veterinary Medicine (7th Edition). English Language Book Soceity).

McIntosh, G.H. (1978). Urolithiasis in animals. (Cited in Wang, X., Lin, C., Zhang, G., Huang, K., Gao, J. and Shen, X. (1997). Chemical composition and microstructure of uroliths and urinary sediment crystals associated with the feeding of high-level cotton seed meal diet to water buffalo calves. *Res. Vet. Sci.* **62**(3): 275-280.

McIntosh, G.H., Pulsford, M.F., Spencer, W.G. and Rosser, H. (1974). A study of urolithiasis in grazing ruminants in south Australia. *Aust. Vet. J.* **50**(8): 345-350.

*Mayer, H. (1972). An anomaly of renal phosphorus excretion in sheep. *Nutr. Abstr. Rev.* **43**(5): 378.

Mercy, A.D. (1979). Studies on the nutrient requirements of kids. M.V.Sc. Thesis submitted to Faculty of Veterinary and Animal Sciences, Kerala Agricultural University, Mannuthy, Thrissur.

Mercy, A.D., Sivaraman, E., Kunjikutty, N. and Annamma Kurian (1981). Studies on the growth rate, feed efficiency and digestibility coefficients of nutrients in Alpine-Malabari crossbred kids. *Kerala J. Vet. Sci.* **12**(1): 164-170.

Mohinder Singh, Sharma, S.K., Vashinsth, N.K. and Varashnay, A.C. (1995). Rupture of urinary bladder due to obstructive urolithiasis in ram - a case report. *Indian Vet. J.* **72**(4): 390-391.

*Nottle, M.C. (1976). Urinary sediments in sheep feeding on estrogenic clover. 4. Indigotin and indirubin in some sediments and calculi. *Nutr. Abstr. Rev.* **46**(9): 816.

Orsborne, C.A., Kalausner, J.S., Krawier, D.R. and Griffith, D.P. (1981). Canine struvite urolithiasis: Problems and their dissolution. *J. Am. Vet. Med. Assoc.* **179**(3): 239-244.

Orskov, E.R. and Robinson, J.J. (1981). Urolithiasis in lambs. *Vet. Rec.* **109**(5): 107.

*Packett, L.V. and Coburn, S.P. (1965). Urine proteins in nutritionally induced ovine urolithiasis. *Nur. Abstr. Rev.* **35**(2): 558.

Packett, L.V. and Hauschild, J.P. (1964). Phosphorus, calcium and magnesium relationships in ovine urolithiasis. *J. Nutr.* **84**(2): 185-189.

Packett, L.V., Lineberger, R.O. and Jackson, H.D. (1968). Mineral studies in ovine phosphatic urolithiasis. *J. Anim. Sci.* **27**(5): 1716-1721.

Petersson, K.H., Warner, R.G., Kallfelz, F.A. and Crosetti, C.F. (1988). Influence of magnesium, water and sodium chloride on urolithiasis in veal calves. *J. Dairy Sci.* **71**(12): 3369-3377.

*Poole, D.B.R. (1990). Observation on the role of magnesium and phosphorus in the aetiology of urolithiasis in male sheep. *Nutr. Abstr. Rev.* **60**(7): 562.

Poonguzhali, K. and Varalakshmi, P. (1992). Effect of banana stem juice on biochemical changes in liver on normal and hyper - oxaluric rats. *Indian J. Exp. Bio.* **30**(5): 440-442.

Puntriano, G.O. (1954). Urinary calculi in livestock - a new concept of this etiology and possible prevention with hyaluronidase. *J. Am. Vet. Med. Assoc.* **124**(922): 55-62.

Ranjhan, S.K., Mohanty, G.O. and Talapathra, S.K. (1965). Hard water and urinary calculi in buffalo calves. *Indian Vet. J.* **42**(1): 182-186.

Read, W.K. (1983). Renal medullary crest necrosis associated with phenylbutazone therapy in horse. *Vet. Pathol.* **20**(6): 662-669.

Rice, D.A. and McMurray, C.H. (1981). Urolithiasis in calves. *Vet. Rec.* **109**(4): 88.

Robbins, J.D., Kunkel, H.O. and Crookshank, H.R. (1965). Relationship of dietary mineral intake to urinary mineral excretion and incidence of urinary calculi in lambs. *J. Anim. Sci.* **24**(1): 76-82.

*Robertson, W.G., Peacock, M., Marshall, R.W., Marshall, D.H. and Nordin, B.E.C. (1977). Saturation inhibition index as a measure of risk of calcium oxalate stone formation in the urinary tract. *Nutr. Abstr. Rev.* **47**(8): 808.

Romanowaski, R.D. (1965). Biochemistry of urolith formation. *J. Am. Vet. Med. Assoc.* **147**(12): 1324-1326.

Shyama (1994). Effect of dried spleen as growth stimulator in kids rations. M.V.Sc. thesis submitted to Faculty of Veterinary and Animal Sciences, Kerala Agricultural University, Mannuthy.

Singh, P.P. and Hussain, F. (1992). Effect of dietary salt supplementation on urinary profile of Guinea pig (*Cavia porcellus*). *Indian J. Exp. Bio.* **30**(5): 443-444.

Singh, P.P., Prasoonhada, Narula, I.M.S. and Gupata, S.K. (1987). *In vivo* effect of Tamarind (*Tamarindus indicus* L.) on urolith inhibitory activity in urine. *Indian J. Exp. Bio.* **25**(12): 863-865.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical methods. 4th edn. The Iowa State, College Press, Hiner, Iowa, Oxford and IBH Publishing Co., Calcutta.

Stewart, S.R., Emerick, R.J. and Pritchard, R.H. (1990). High dietary calcium to phosphorus ratio and alkali forming potential as factor promoting silica urolithiasis in sheep. *J. Anim. Sci.* **68**(2): 498-503.

Stewart, S.R., Emerick, R.J. and Pritchard, R.H. (1991). Effect of dietary ammonium chloride and variations in calcium to phosphorus ratio in silica urolithiasis in sheep. *J. Anim. Sci.* **69**(8): 2225-2229.

Swingle, K.F. and Marsh, H. (1956). Vitamin A deficiency and urolithiasis in range cattle. *Am. J. Vet. Res.* **17**(6): 415-423.

Thomas, C.T., Devasia, P.A., Kunjikutty, N. and Nandakumaran, N. (1976). Studies on feeding of goats. III Evaluation of nutritive value of rain tree (*Enterlobium saman* or *Samanae saman*) fruit meal for growth. *Kerala J. Vet. Sci.* 7(1): 13-19.

Udall, R.H. (1959). Studies on urolithiasis - the control by force feeding sodium chloride. *Am. J. Vet. Res.* **20**(6): 423-425.

Udall, R.H. (1962). Studies on urolithiasis 5. The effect of urinary pH and dietary sodium chloride on the urinary excretion of protein and the incidence of calcinosis. *Am. J. Vet. Res.* **23**(15): 1241-1245.

Udall, R.H. and Chow, F.H.C. (1965). Studies on urolithiasis: 7. Effect of sodium, potassium or chloride ions in the control of urinary calculi. *Cornell Vet.* **55**(6): 538-544.

Udall, R.H. and Jensen, R. (1958). Studies on urolithiasis II. The occurrence in feedlot lambs following implantation of Diethylstilbesterol. *J. Am. Vet. Med. Assoc.* **133**(10): 514-516.

Udall, R.H., Segar, C.L. and Chow, F.H.C. (1965). Studies on urolithiasis: 6. The mechanism of action of sodium chloride in the control of urinary calculi. *Cornell Vet.* **55**(3): 198-203.

*Van Reen, R., Simmons, W.K. and Jenkin, L.J.(Jr.). (1965). Urolithiasis in the rat. 4. Influence of amino acid supplementation on the occurrence of citrate calculi. *Nutr. Abstr. Rev.* **35**(2): 449.

Vipperman, P.E., Preston, R.L., Kintner, L.D. and Pfander, W.H. (1969). Role of calcium in the nutritional etiology of a metabolic disorder in ruminants fed a high grain ration. *J. Nutr.* **97**(4): 449-462.

Waltner-toews, D. and Meadows, A.A. (1980). Urolithiasis in a herd of beef cattle associated with oxalate ingestion. *Can. Vet. J.* **21**(2): 61-62.

Winer, J.H. (1959). Practical value of analysis of urinary calculi. (Cited in Danowski, T.S. (1962). Clinical Endocrinology, Vol.III. The Williams and Wilkins Company, U.S.A.).

*Yano, H. and Kawashima, R. (1977). *In vitro* study on mechanism of ammonium chloride on the prevention of urolithiasis in fattening cattle. *Nutr. Abstr. Rev.* **47**: 358.

*Yano, H., Sakurai, S. and Kawashima, R. (1977). Effect of dietary calcium and phosphorus levels on the incidence of urinary calculi and on urine and serum mineral levels in wethers. *Nutr. Abstr. Rev.* **47**: 358.

* Originals not consulted

EFFICACY OF AMMONIUM CHLORIDE AND HORSE GRAM *(Dolichos biflorus)* EXTRACT ON THE AMELIORATION OF UROLITHIASIS IN GOATS

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ABSTRACT

The efficacy of ammonium chloride and horse gram (*Dolichos biflorus*) extract on the amelioration of urolithiasis in goats ware evaluated by using 18 male Malabari goats of 9 to 12 months old, maintained for an experimental period of 84 days. Goats were divided into three groups (I, II and III) of six animals each and were fed individually at maintenance level with high magnesium basal calculogenic ration containing 1.194 per cent calcium, 0.578 per cent phosphorus, 1.202 per cent magnesium (ration A) alone, fortified with ammonium chloride at the rate of one per cent in the ration (ration B) or with supplemented horse gram extract at the rate of one litre per animal per day (ration C) respectively. Grass and concentrate were fed at 1:4 ratio and drinking water provided *ad libitum* throughout the experiment. Body weight gain, dry matter intake, feed efficiency and protein efficiency were not significantly altered by the dietary treatments, but goats fed on supplemental ammonium chloride (group II) had a trend towards better weight gain, feed and protein efficiency. No significant difference observed among the three groups with regard to TEC, TLC, haemoglobin, and plasma protein. Elevated dietary magnesium in the diet (ration A) caused significant decrease in serum

calcium ($P<0.01$), significant increase in serum phosphorus and magnesium ($P<0.01$). There were increased excretion of urine calcium ($P<0.05$), significant increase in urine phosphorus and magnesium ($P<0.01$) in group I, when compared to groups II and III. Supplemental ammonium chloride and horse gram extract caused significant rise in serum calcium, significant reduction in serum phosphorus and magnesium and significant reduction in urine calcium, phosphorus and magnesium, whereas horse gram extract was less effective than ammonium chloride in this regard. Both the dietary treatments had no significant influence on urinary nitrogen excretion and digestibility of dietary calcium, phosphorus magnesium and nitrogen. Supplemental ammonium chloride caused significant increase in per cent retention of calcium ($P<0.01$), and phosphorus ($P<0.05$) but supplemental horse gram extract had only a tendency to increase per cent retention of calcium and phosphorus. Both the dietary treatment had no influence on nitrogen retention and supported nitrogen retention during the progress of experiment. Magnesium retention has increased drastically due to high dietary supplementation of magnesium in all the three groups and neither supplemental ammonium chloride nor horse gram extract had any significant influence on per cent retention of magnesium. Clinical signs of obstructive urolithiasis were not observed in any of the goats maintained on three experimental groups. Goats in group I

exhibited severe crystalluria, had numerous visible sand like rudimentary calculi in the kidney and had severe gross and histopathological changes. Goats fed with supplemental ammonium chloride showed reduction in urine pH, increased urine volume had not found to have any calculus material in the kidney and had mild gross and histological changes in the kidney and bladder. Goats fed on supplemental horse gram extract showed reduction in intensity of crystalluria, had few calculi materials in the kidney with gross and histological changes in the kidney and bladder comparable to group I. On chemical analysis, the calculi were found to contain magnesium, phosphate and ammonia. The present investigation conclude that supplementation of ammonium chloride prevented the calculi formation possibly due to increased excretion of chloride ions in the urine. Supplemental horse gram extract had not prevented calculi formation when fed along with high magnesium calculogenic ration in goats whereas, had a tendency to prevent the incidence of urinary calculi perhaps due to its diuretic effect.