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FEED SUPPLEMENTATION BY AMMONIUM CHLORIDE AND/OR VITAMIN "A" TO CHECK FORMATION OF URINARY CALCULI IN SHEEP

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ABSTRACT

This study was carried out to estimate the effect of ammonium chloride and/or vitamin "A" supplementation on checking formation of urinary calculi in rams. Twenty four rams were used in four equal groups each of six animals. The first group was supplemented with ammonium chloride (5 gm/kg of diet), the second group was supplemented with vitamin "A" (3000 IU/kg diet), the third group was supplemented by ammonium chloride (5 gm/kg diet) plus vitamin "A" (3000 IU/kg diet) while the fourth group remained as control. Ration, water and serum were analysed for their contents of minerals related to urinary calculi formation. The results showed that there were mineral's imbalances in the basic ration and, as well in the blood serum of the control group. Supplementing rams with ammonium chloride and/or vitamin "A" improved mineral content and reduced the imbalances in calcium : phosphorus ratio and sodium, potassium and magnesium concentration in blood serum. Determination of pus cells, epithelial cells, red blood cells and triple phosphate crystals in the urine revealed that these parameters were decreased after supplementation with vitamin "A" and ammonium chloride, mostly through improving Ca^{++} : P^{---} ratio in blood.
Key words : Sheep, ammonium chloride, vitamin A, urinary calculi.

INTRODUCTION

Urolithiasis is an awful disease in ruminant animals since it results in death or lowering their performance. The disease is a result of formation of stones or calculi in the kidney or urinary bladder causing obstruction of urine excretion (Church, 1984). Urethral obstruction resulting from urolithiasis is a serious and potentially life threatening problem in small ruminants (Khamis and Saleh, 1970). Urolithiasis has been reported to become frequent in male ruminants under intensive production and increasing use of concentrate rations. Such rations are imbalanced in their mineral content and deficient of vitamin A being calculigenic rations. The incidence of urinary calculi has been increased by feeding grain diets high in phosphrous and low in calcium (Jensen and Swifts, 1988). Khamis and Saleh (1970) reported that higher incidence of urolithiasis in summer in Egypt may be partly attributed to the insufficient carotene intake by male ruminants in this season.

Sheep and goats are reported as more susceptible ruminants to urolithiasis. Dietary intake of calcium, phosphorus, magnesium, sodium and potassium appear to play a major role in the incidence of calculosis in lambs. Ammonium chloride or ammonium sulphate added to the complete diet at 0.5 percent had been used successfully to prevent urinary calculi formation. Ammonium chloride appears to be more effective than ammonium sulphate (Crookshank, 1970). Ammonium chloride has been used by drenching at a level of 7-14 gm per sheep for 3 to 5 days. Reasonable success has been

achieved with this practice, as animals were still able to pass small amounts of urine. Injection with a smooth muscle relaxant is helpful in these cases. Supplementary vitamin "A" has been reported to alleviate complications of urinary calculi in sheep. Its effect is to keep healthy epithelium of urinary system (El-Attar *et al.*, 1989). Post mortem examination of dead rams in Borg El-Arab Research Station, in Egypt, during 1998 and 1999 showed that more than 60% of the mortality cases were due to urinary calculi during summer feeding period.

The objective of this study was to investigate the value of supplementation with ammonium chloride and/or vitamin "A" in reducing expected urinary calculi formation during summer season with feeding high concentrate rations.

MATERIALS AND METHODS

The experiment was carried out at Borg El-Arab Research Station, Animal Production Research Institute, for a period of 4 months during May - August 2000. Twenty four rams, as available in the Station, divided into four equal groups, each of six animals were used during summer season. The rams age ranged between 2 to 4 years with live body weight between 50 and 70 kg. The animals were fed on Berseem (*Trifolium alexandrinum*) ad lib. and 0.5 kg concentrate mixture/head/day, before starting the experiment. During the experiment, daily allowance of concentrate feed was 3% of live body weight, as group feeding. The concentrate mixture contained (7% decorticated cotton seed meal, 4% soyabean meal, 20% wheat bran, 20% rice bran, 23% ground maize, 15% molasses, 8% rice gluten, 2.5% limestone and 0.5% common salt). The TDN and DCP values were 56.5 and 11%, respectively. Animals were also provided with Berseem hay as a source of roughage, 1% of body weight. Drinking water was available to the animals allover the day. The first group of animals was kept as, control, the other groups were treated as: (T1) supplemented with 5 gm ammonium chloride/ kg of diet, (T2) supplemented with vitamin "A" (3000 IU/kg diet) and (T_3) supplemented with 5 gm ammonium chloride + 3000 IU vitamin "A"/kg diet. Ammonium chloride and vitamin "A" doses were added as recommended by Crookshank (1970). Ammonium chloride was daily added to concentrate allowance for the treated animals, fully consumed. Vitamin "A" capsules of 50,000 IU each was drenched once every 8 days throughout the experimental period, where the study lasted for 4 months.

Monthly blood samples were collected from the jugular vein of the animals in the morning before access to feed and water. Separated serum was analysed for calcium, inorganic phosphorus, magnesium, sodium and potassium concentrations. Calcium and sodium were tested by kits from Stanbio Laboratory, Inc. Inorganic phosphorus and magnesium were determined by spectro-photometry using kits from Bio-Al-CHE-MA, Kit, Czech Rep. Potassium was determined using Bio-Analytics, Kit, USA.

Animals were enforced to urinate by putting a wetted cloth for temporarily closing nose and mouse of sheep to stop breathing for 10 seconds (Youssef,

1999), then animal urinate and urine was collected in polyethylene bags. Urine samples were collected once monthly from each animal. Fifteen ml of each urine sample was directly centrifuged at 1000 rpm for 5 min. The deposited material was smeared over a glass slide covered with a thin glass cover to estimate frequencies of pus cells, epithelial cells, triple phosphate crystals and RBC's. Microscopic examination was carried out at (10x40) according to Coles (1986). Concentrate feed mixture and berseem hay were analyzed according to AOAC (1970) procedures, drinking water was analyzed for minerals contents in El-Naser Lel Mallahat, Company, Egypt.

The data were statistically analyzed using the General Linear Model procedure of SAS (1986). The statistical model included treatment, period and their one-way interactions (Steel and Torrie, 1980). Duncan's New Multiple Range test was used to compare means of individual animals.

RESULTS AND DISCUSSION

Data presented in Table (1) show the chemical and mineral composition of the experimental ration. It could be noticed that there were an imbalance between the minerals contents in the diet. Calcium (Ca) : phosphorus (P) ratio was 0.139 : 1 in concentrate mixture and 2.8 : 1 in berseem hay. The wide range between Ca concentration and P in the concentrate mixture help to form urinary calculi as stated by Crookshank (1968). A narrow calcium to phosphorus ratio (<2:1) may contribute to an increased incidence of urinary calculi in intact and castrated male sheep (NRC, 1981). Also, this case was found to be due to unbalances between sodium and potassium concentrations in the diet. Potassium content in the diet was reported to have curvilinear relation with urolith formation. In wethers fed calculogenic diets, maximum urolithiasis occurred with 0.64 percent potassium in the diet and decreased at levels above and below that (Lampercht *et al.*, 1969). Sodium functions in maintenance of osmotic pressure; regulation of the acid base balance and control of water metabolism in tissues are related to urinary calculi formation.

Table (1) : Chemical composition and mineral contents of the experimental ration.

Item	Concentrate mixture	Berseem hay
Chemical composition (%)		
Dry matter (DM)	86.9	78.7
Crud protein (CP)	16.1	14.3
Crud fat (Fat)	4.1	1.1
Crud fiber (CF)	8.7	36.4
Ash	6.1	15.1
Nitrogen free extract (NFE)	65.0	33.0
Minerals contents (%)		
Calcium (Ca)	0.24	0.41
Phosphorus (P)	1.71	0.14
Sodium (Na)	1.58	1.51
Potassium (K)	0.13	0.46

Table (2) : Chemical analysis of water used in the experiment.

Item	
pH	7.81
Total salt	0.375 g/L
Calcium	0.048 g/L
Phosphorus	0.003 g/L
Sodium	0.178 g/L
Magnesium	0.078 g/L

Results presented in figure (1) and table (3) indicate that ammonium chloride supplementation alone or with vitamin "A" increased significantly ($p<0.01$) serum calcium level by 18.6 and 17.6% for T_1 and T_3 respectively, from control. However, animals in control group showed drop in serum calcium level during the experimental period, where it decreased from 10.17 mg/100ml at the beginning to 8.17 mg/100 ml at the end of the experiment (4 months). These results are in agreement with those found by Hussien *et al.* (1988) and Stewart *et al.* (1991), they reported that ammonium chloride caused significant increase in serum calcium, preventing urolithiasis in the lambs. Whereas, Stewart *et al.* (1991) reported that ammonium chloride decreased urinary pH and increased urine volume. Also, Khamis *et al.* (1979) and Kallfelz *et al.* (1987) showed that high blood serum calcium has been reported as having a protective action against urolthiasis.

Results presented in figure (1) and table (3) indicate that blood serum inorganic phosphorus showed a sharp increase after one month of treatment for the control group, with gradual increase from month 2 to the end of the experiment (month 4). The group's means of blood serum inorganic phosphorus showed significant ($p<0.01$) decreases as a percentage of 24.0%, 11.5% and 30.0% for T_1, T_2 and T_3, respectively, from the control level. These results are in agreement with those reported by Youssef (1999) and Hussien *et al.* (1988), where, they reported that ammonium chloride caused a significant decrease in serum phosphorus, whereas, high blood serum phosphorus was reported to be associated with high incidence of urinary calculi in lambs (Youssef, 1999). El-Attar *et al.* (1989) found that the incidence of urinary calculi in rams was accompanied with a decrease in the serum contents of carotenoids, vitamin "A" and calcium level and increase in inorganic phosphorus, creatinine and alkaline phosphates. Also, Crookshank *et al.* (1967) and Agag *et al.* (1990) stated that high level of dietary phosphorus resulted in significant high serum phosphorus level associated with high urolithiasis.

The concentration of serum magnesium decreased significantly ($p<0.01$) during the experimental period (Table 3). Animals in T_1 showed decrease in concentration of serum magnesium from 3.24 to 2.79 mg/100ml at the 4th month (Fig. 1). Animals in T_3 had a decreased level of serum magnesium from 3.20 to 2.47 mg/100ml in the 2nd month, followed by an increase its level to reach 3.01 during the 4th month of the experiment, however it was still far below the control. On the other hand, animals of T_2 showed slight insignificant increase (Table 3) from 3.01 to 3.20 mg/100ml in the 4th month of

the experiment. However, the overall mean values decreased by about 22.0%, 13.0% and 26.0% in animals of T_1, T_2 and T_3 from that of the control which showed relatively high blood serum magnesium from 3.40 to 4.18 mg/100ml at the 4th month. The present results are in agreement with the findings of Christopher (1984), Peterson *et al.* (1988) and Fahmy *et al.* (1999) who reported that high serum magnesium was associated with high incidence of urinary calculi. Also, Hussien *et al.* (1988) reported that addition of ammonium chloride to the basal ration significantly decreased serum magnesium preventing urolithiasis.

The changes in blood serum sodium and potassium due to treatments are presented in figure (2) and table (3). The results show that animals in control group or that treated with ammonium chloride alone (T_1) had higher sodium concentration than that treated with vitamin "A" only (T_2) or vitamin "A" with ammonium chloride (T_3). This may be due to the effect of vitamin "A" in (T_2) and (T_3). Youssef (1999) mentioned that vitamin "A" is reported to maintain healthy kidney epithelium. Potassium levels were decreased during the 2nd month of the experiment in control, T_2 and T_3 groups then increased insignificantly (Table 3) during the 3rd and 4th month of the experiment. Treatment 1 caused increase in the 2nd month followed by decrease in the 3rd and 4th month. The overall mean value of potassium was significantly ($p<0.01$) higher in T_1 than that of control, T_2 and T_3. The results about sodium and potassium are in agreement with the finding of Youssef (1999) in ram lambs. Bushman *et al.* (1968) found that addition of ammonium chloride had no significant effect on blood serum sodium and potassium levels in lambs. Moreover, Crookshank *et al.* (1967) suggested that blood serum sodium or potassium levels had no relation with the development of urolithiasis in the lambs.

Results presented in figure (3) and table (3) show that urine sediment of the control group contained more pus, epithelial, red blood cells and triple phosphate crystals. Dietary supplementation by ammonium chloride with vitamin A significantly ($p<0.01$) decreased urinary pus cells, epithelial cells, red blood cells and triple phosphate crystals. The decreasing percentages were 52, 62, 50 and 68%, respectively, as compared with control. Moreover, animals treated with ammonium chloride alone (T_1) or vitamin "A" alone (T_2) showed lowering of pus cells, epithelial cells, triple phosphate crystals and red blood cells but not as in T_3. Agag *et al.* (1988) using microscopic examination of urine sediment of bucks suffering from urine retention revealed increase of pus cells, epithelial cells and triple phosphate crystals. Also, Christopher (1984) reported that urine sediment of 31 clinical cases of urolithiasis contained few pus and epithelial cells only in some cases. Youssef (1999) concluded that ammonium chloride was effective in reducing the triple phosphate crystals count but it was not effective to keep healthy renal epithelium. The advantages to keep healthy epithelium and to reduce triple phosphate crystals were achieved by treating sheep with both vitamin "A" and ammonium chloride. It is of interest to notice from table (3) that there was significant ($p<0.01$) treatment x period interaction in all parameters studied except potassium concentration.

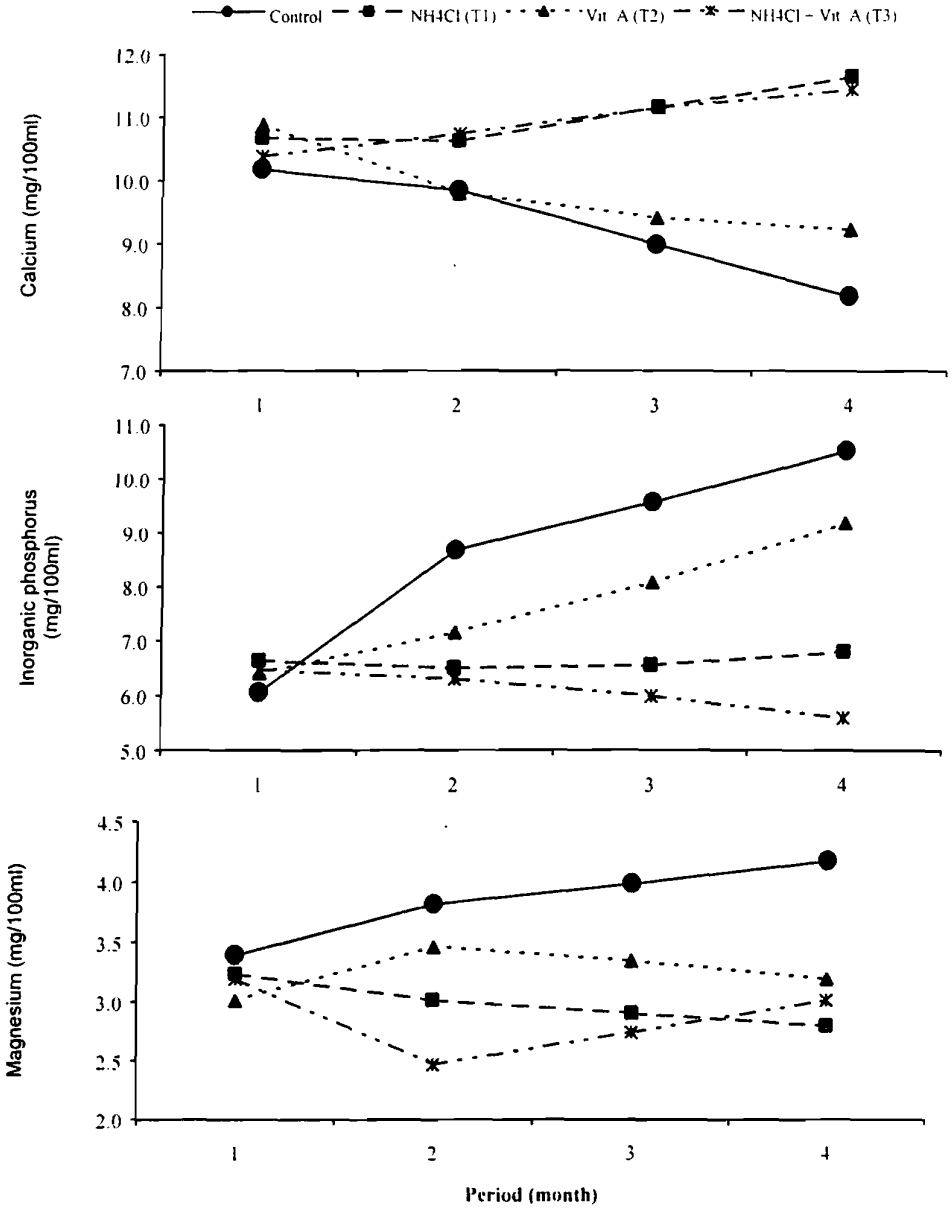

Fig (1) : Variations in blood serum calcium, inorganic phosphorus and magnesium concentration (mg/100ml) during the experimental period, by the four treatments.

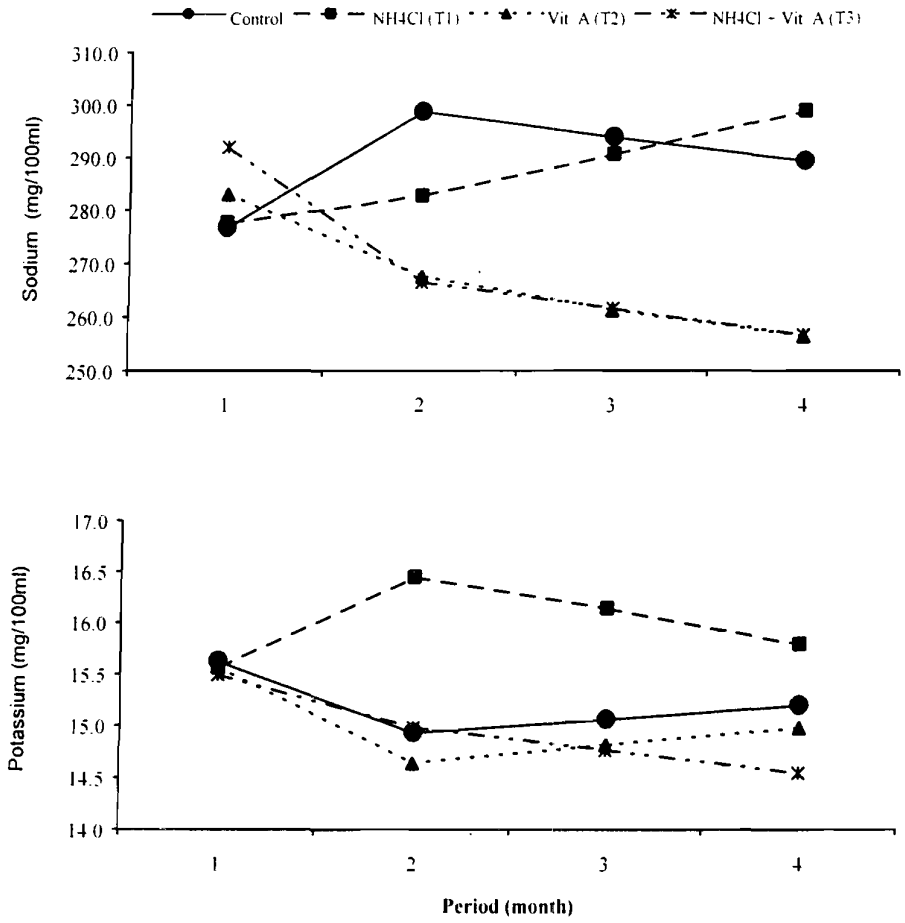

Fig (2) : Variations in blood serum sodium and potassium concentrations (mg/100 ml) during the experimental period, by the four treatments.

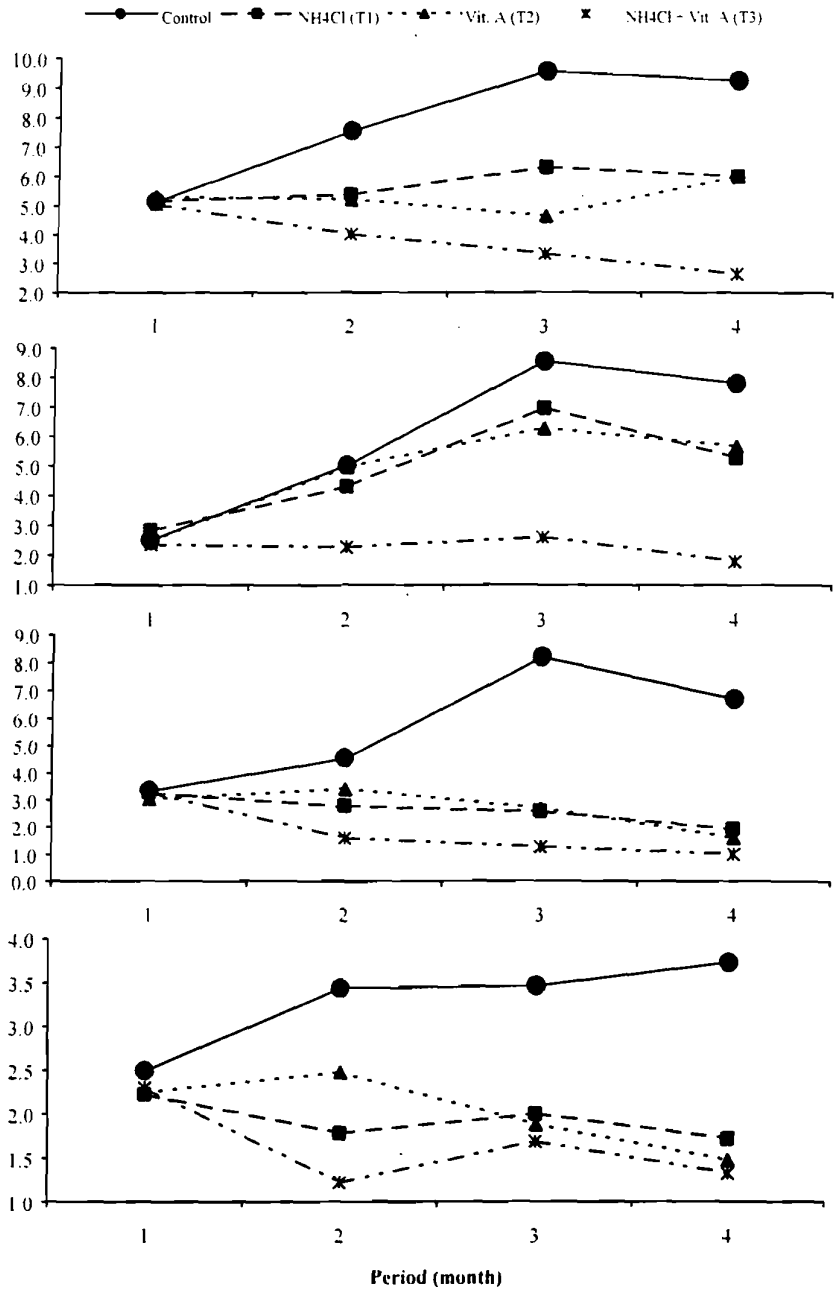

Fig (3) : Average of pus cells, epithelial cells, triple phosphate crystals and red blood cells (count x 400) in the urine sediment during the experimental period, by the four treatment.

Table (3) : Analysis of variance for the effect of treatment and period and their interaction on the blood serum concentration of : calcium, Inorganic phosphrus, magnesium, sodium and potassium alongside counts of : pus cells, epithelial cells, triple phosphate crystals and red blood cells in urin.

S.O.V.	d.f	Calcium	Inorg. Phospho.	Magnesium	Sodium	Potassium	Pus cells	Epith. Cells	Triple phosphate crystals	RBCs
Treatment (T)	3	17.10**	32.55**	4.64**	3357.94**	5.47**	68.89**	526.46**	70.10**	12.76**
Peiod (P)	3	0.81	11.41**	0.05	215.61	0.89	3.55**	55.66**	3.18**	0.28
T x P	9	3.31**	6.74**	0.56**	1039.66**	0.85	10.25**	8.65**	12.22**	1.46**
Error	80	0.59	0.24	0.04	172.89	0.47	0.40	0.38	0.22	0.23

** Significant differences ($p<0.01$) in the mean values as presented in figures 1-3.

After four months of treatment the rams acquired the cases shown in the following table :

Treatment	Blood serum	Urine sediment smear (count x 400)			
	Calcium : Phosphorus	Pus cells	Epith. Cells	Triple phos. crystals	RBCs
Control	1.07 : 1.00	7.86	5.98	5.68	3.28
T_1	1.66 : 1.00	5.71	4.85	2.63	1.93
T_2	1.28 : 1.00	5.29	4.85	2.69	2.02
T_3	1.80 : 1.00	3.76	2.25	1.79	1.63

It is clear in this table that addition of ammonium chloride alone (T_1) or with vitamin "A" (T_3) increased serum calcium level and decreased serum inorganic phosphorus. Also, addition of ammonium chloride and/or vitamin "A" maintained low counts of pus cells, epithelial cells, triple phosphate crystals and red blood cells compared with the control group, but significantly low counts occurred with (T_3). So, addition of ammonium chloride and vitamin "A" was more effecting in checking formation of urinary calculi in sheep alongside maintenance of normal renal epithelium during summer season with feeding such concentrate rations.

REFERENCES

Agag, B.I.; M.A.M. Hafez; E.E.A Safwat and N.M. Aly (1988). Phosphatic urolithiasis as a field problem in goat farms. J. Egypt Vet. Med. Asso., 48: 25.

Agag, B.I.; M.H.M. Shaker; N.V. Bishara; S.M. Mousa and S.I. Hegazi (1990). Report on field cases of obstructive urolithiasis in buffalo-calves. Egyptian. J. Comp. Path. Clin. Path., 3:257.

Association of Offical Agricultural Chemists (A.O.A.C.) (1970). Official Methods of Analysis, (2nd E.d.) Washington, D.C.

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

Christopher, K.J. (1984). Some aspects of urolithiasis in cattle. Veterinarski Arhiv., 54: 239.

Church, D.C. (1984). Digestive Physiology and Nutrition of Ruminants. Volume 2. Nutrition. Ruminant Nutritionist O. & B. Books, Inc. U.S.A.

Coles, E.H. (1986). Veterinary Clinical Pathology (4th Ed.) W.B. Saunders Company, Philadelphia, U.S.A.

Crookshank, H.R. (1968). Prevention and control of urinary calculi in sheep. pp. 162-172 in Proc. Symp. On sheep diseases and health, University of California, Davis.

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

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

El-Attar, H.M.; B.I. Agag, H.A. Hassanein: E.E. Safwat and M.A. Eid (1989). Urolithiasis syndrom as a field observation in rams. The veterinarian, 7: 54.

Fahmy, Lotfia, S.; Y. Khamis; A.A. Hegazy; H.M. Gohar and A.A. Shamaa (1999). Effect of high dietary levels of calcium, phosphorus and magnesium on urolithiasis in sheep. Workshop on Livestock and Drought : Policies for Coping with Changes. Cairo, Egypt, p. 159.

Hussien, M.; S.A. Ramdan; H.M. Gohar and Y.A. Radwan (1988). A field trial to prevent sheep urolithiasis by the use of ammonium chloride, calcium chloride, sodium chloride and calcium carbonate. Alex. J. Vet. Sci., 1: 369.

Jensen, R. and Swifts (1988). Diseases of Sheep (3rd Ed.) Lea and Febiger. Philadelphia, USA.

Kallfelz, F.A.; A.S. Ahmed; R.J. Wallace; B.H. Sasangka and R.G. Warner (1987). Dietary magnesium and urolithiasis in growing calves. Cornell Veterinarian, 77:33.

Khamis, M.Y.; A.H. Said and H.M. Gohar (1979). The effect of dietary [illegible] in comparison with calculogenic diet. Egypt. Vet. Med. Asso, 14th ARAB Vet. Congress, Cairo, Egypt.

Khamis, M.Y. and M. Saleh (1970). Urolithiasis syndrome in ruminants. Egypt. Vet. Med. J., 18: 241.

Lamprecht, W.O.; J.G. Darroch and H.R. Crookshank (1969). Statistical analysis of dietary mineral intake and the occurrence of urolithiasis in sheep. J. Anim. Sci., 28: 386.

N.R.C. (1981). Nutritional Requirement of Sheep. National Acadmy of Science, National Research Council, Washington, DC., U.S.A.

Peterson, K.H.; R.G. Warner; F.A. Kallfelz and C.F. Crosetti (1988). Influnce of magnesium, water and sodium chloride on urolithiasis in veal calves. J. Dairy Sci., 71: 3369.

SAS (1986). User's Guide, Statistical Analysis System. Var. 5, Ed. SAS Institute, Cary, N.C.

Steel, R.G.D and J.H. Torrie (1980). Principles and Procedures of Statistics (2nd Ed.). Tosho Printing Co., LTD. Tokyo, Japan.

Stewart. S.R.; R.J. Emerick and R.H. Ritchard (1991). Effect of dietary ammonium chloride and variation in calcium to phosphorus ratio on silica urolithiasis in sheep. J. Anim. Sci., 69 : 2225

Youssef, H.H.H. (1999). Effect of dietary ammonium chloride and vitamin A on urolithiasis in sheep fed high concentrate ration. M.Sc. Thesis. Fac. Agric., Cario. Univ. Egypt.

تأثير إضافة كلوريد الأمونيوم وفيتامين " أ " أو هما معا علي معارضة تكوين الحصوات البولية في الأغنام

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أجري هذا البحث علي ٢٤ حيوان من ذكور الأغنام، وقسمت إلي أربعة مجموعات كل مجموعة من ستة حيوانات وذلك لدراسة أثر استخدام كلوريد الأمونيوم أو فيتامين أ أو كلاهما معا في منع تكوين الحصوات البولية. أخذت عينات من الدم وكذلك من العلف والمياه لتقدير بعض المعادن وكذلك أخذت عينات من البول لتقدير نسبة الكرات الدموية الحمراء والخلايا الطلائية وبلورات ثلاثي الفوسفات. وجد أنه يوجد خللا واضحا بين نسب المعادن في الأعلاف ما بين الكالسيوم والفوسفور والصوديوم والبوتاسيوم. استخدم كلوريد الأمونيوم وحده في المعاملة الأولي بمعدل ٥جم/كجم وزن عليقة أو استخدام فيتامين أ بمعدل ٣٠٠٠ وحدة دولية في المعاملة الثانية واستخدام كلوريد الأمونيوم ٥جم/كجم عليقة + ٣٠٠٠ وحدة دولية في المعاملة الثالثة بينما بقيت مجموعة كونترول. عند استخدام كلوريد الأمونيوم وحده أو مع فيتامين "أ" أدي إلي تصحيح الخلل ما بين نسب المعادن في الدم ونقص في مستوي الفوسفور والماغنسيوم وزيادة في مستوي الكالسيوم مقارنة بالكونترول. كانت أفضل المعاملات هي المعاملة الثالثة بكلوريد الأمونيوم وفيتامين "أ" والتي أحدثت توازن ما بين نسب المعادن في الدم. نقصت نسبة الكرات الدموية الحمراء والخلايا الطلائية وكذلك بلورات ثلاثي الفوسفات في بول الحيوانات خاصة في المعاملة الثالثة. ومن ذلك يتضح أن المعاملة بكلوريد الأمونيوم ٥جم/كجم عليقة + ٣٠٠٠ وحدة دولية فيتامين " أ " مفيدة للأغنام خاصة في الفترة التي يتعرض فيها الحيوان لتكوين الحصوات البولية صيفا نتيجة تناول علائق عالية في المركزات.