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Full Length Research Paper

Clinical, mineral and haemato biochemical studies of urolithiasis in weaner lambs

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Retrospective study on necropsy data pertaining to urolithiasis in sheep was conducted to observe the epidemiological profile in semi-arid Rajasthan. Subsequently in year 2008, a total of 27 weaner lambs comprised of 23 clinically affected with urolithiasis and 4 clinically healthy lambs were undertaken to study the mineral and haemato biochemical profile in an attempt to find out the probable cause of the menace. During the period, out of 23 diseased lambs 11 were and they subjected to post-mortem examination for confirmation of urolithiasis. The confirmed urolith cases had slightly higher Hb, PCV, TEC, RBC, WBC and epithelial casts in urine, and significantly higher serum urea creatinine, magnesium and copper compared to suspected and healthy animals. The study revealed that abnormal calcium–phosphorus ratio is predisposing the animals to urolithiasis; however; other interrelated factors needs to be explored to formulate the effective preventive measures.

Key words: Urolithiasis, sheep, hematology, biochemical profile, mineral profile.

INTRODUCTION

Urolithiasis has been attributed to be the fifth most prevalent cause of death in feedlots (Singh et al., 1981). It is a common disease of ruminants caused by formation calculi in the urinary tract with subsequent blockage to urine outflow consequently leading to uremia and death. Crystallized minerals (uroliths) are primarily responsible for urinary tract blockage. It appears to affect equally both sexes; however, urinary blockage is an important problem only in males because of the anatomical conformation of their urinary tract (Larson, 1996; Radostits et al., 2000). Urinary calculi formation usually results from a combination of physiologic, nutritional and management factors. It is mainly attributed to excessive or imbalanced intake of minerals (McIntosh, 1978; Larson, 1996; Radostits et al., 2000). A calcium-phosphorus imbalance results in high urinary phosphate excretion, which is an important factor in the genesis of phosphate calculi. Numerous additional factors like heavy concentrate-low roughage diets, limited water intake, deprivation of water or dehydration, urine alkalinity, mineralized artesian water, alkaline water supplies, excess of sodium bicarbonate in the diet, vitamin imbalances e.g. hypo vitaminosis A and hyper vitaminosis D and high-protein rations have been incriminated as contributing causes for the development of phosphate calculi with resultant obstructive urolithiasis (Radostits et al., 2000). Fatality rate of urolithiasis in livestock due to rupture of the urethra or urinary bladder and the economic impact of this condition has been extensively reported in field and slaughter-house studies developed in many countries (Gasthuys et al., 1993; Radostits et al., 2000). Since the last few years clinical incidence and death due to urolithiasis particularly in weaner male lambs was increasing at Central Sheep and Wool Research institute (CSWRI), Avikanagar. Thus, the present study was conducted to study the clinical and haemato biochemical profile in them in order to find out possible contributing factors and formulate preventive strategy.

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MATERIALS AND METHODS

Retrospective study on incidence

Necropsy data pertaining to urolithiasis were collected for the period from August 2002 to July 2008 and analyzed to observe the

Table 1. Macro and micro mineral composition of fodder in grazing area and concentrate ration.

Feed	Ca (g%)	P (g%)	Mg (g%)	Zn (ppm)	Cu (ppm)
Fodder	0.42	0.17	0.44	28.60	40.67
Concentrate	2.61	0.49	0.68	86.47	21.42

effect of age, sex, season, sire and breed.

Clinical and haemato biochemical studies

The lambs born during spring, 2008 at CSWRI were taken for study during the period of March – June, 2008. The weaner lambs were fed with concentrate (*ad-lib)*, roughages and allowed for grazing for 3 - 4 h/day. The composition of fodder and concentrate was given in Table 1. The composition of lamb ration was given in Table 2. The source of water for drinking of sheep was deep bore well water. As a preventive measure, all suckling and weaner lambs were given ammonium chloride (at 5.0 g/head) thrice in a week for a period of one month in the summer season. A total of 27 lambs comprised of 23 clinically affected with urolithiasis and 4 clinically healthy lambs were included for study. During the period, out of 23 diseased lambs 11 died and were subjected to post-mortem examination for confirmation of urolithiasis. The urine samples were collected in a sterile container for examination. From the necropsied lambs uroliths were collected.

For haemato biochemical studies, 10 ml of blood was collected from each lamb by jugular venipuncture and 3 ml of blood was transferred into vacuum EDTA coated tubes (Vacutainer ®, BD) and the remaining blood was kept for serum separation. The Hb, PCV, TEC, TLC, DC were estimated with in 2 - 4 h of collection by using standard techniques (Sastry, 1985). Serum creatinine, blood urea nitrogen, alkaline phosphatase, total protein, albumin and total cholesterol were estimated by using the commercial kits (Span diagnostics Ltd, India) and the reading were taken by using Spectrophotometer (Shimadzu UV-160A). The calcium, magnesium, zinc, copper were estimated by using atomic absorption spectrophotometer (Shimadzu, AA – 1600). The phosphorus was analyzed by the method of Fiske and Subbarow (1925). Statistical analysis was performed by ANOVA and DMRT using SPSS ver 15.

RESULTS

Retrospective study on incidence

During the period a total of 109 animals of different breeds died due to urinary tract diseases, involving mainly obstructive urolithiasis, cystitis, urinary bladder rupture and nephritis. The equivalent average death rate (EADR) (Maru et al., 1993) of urolithiasis/1000 animals/day at risk for different breeds were Chokla (0.0032), Malpura (0.0126), Avikalin (0.0148), Bharat Merino (0.0243), Garole x Malpura (0.0283), Malpura x Garole x Malpura (0.0296) and Garole (0.061). The maximum occurrence of 52.29% was recorded in weaner lambs, followed by suckling (27.52%), adult (11.01%) and hoggets (9.17%). Sex - wise analysis revealed highest incidence was in male (92.7%). The monthly pattern showed there was increased incidence from March to July, followed by sporadic incidence in the following months (Figure 1). The seasonal pattern showed increased incidence in summer months (50.46%). In all the cases no sire effect was observed on incidence of urolithiasis.

Clinical and post-mortem observations

The clinical signs exhibited by the suspected lambs varied from anorexia, depression, and restlessness in early stages and in later stage showed bilateral abdominal distention, grinding of teeth, dribbling of urine, arching back, recumbancy. Death ensured 24 - 48 h after the onset of the clinical signs. At necropsy, there was diffused, acute, severe fibrinous peritonitis due to rupture of the urinary bladder and leakage of urine into the abdominal cavity (uroperitoneum). Amorphous granular crystalline, irregular calculi of varying size were recovered from kidney (Figure 2), in the lumen of the urinary bladder and in the urethral lumen. In some cases the urinary bladder was distended and filled with urine (Figure 3) while in majority there was ruptured urinary bladder and uroperitoneum. The mineral profile of urine and uroliths were not performed.

Urinary examination

The urine pH of clinically healthy lambs ranged between 7.9 and 8.1, clinically suspected animals ranged between 8.0 and 8.1 and in confirmed cases ranged between 8.1 and 8.2 revealing non significant variation among all the three groups. Many of the confirmed cases showed presence of protein in urine by Robert's reagent test (Sastry, 1985). But no protein was present in urine of suspected and clinically healthy animals. In many of the clinically confirmed and suspected cases epithelial casts (10 – 12 / hpf), leukocytes (2 - 4 /hpf), RBC (0 - 4 /hpf) were observed during microscopic examination.

Haematological observations

Blood picture exhibited non-significant variations for Hb, PCV, TEC, TLC, monocytes, eosinophils and basophils among different groups of animals and were within the normal range (Sastry, 1985). However, a moderate increase in Hb, PCV, and TEC values were observed in confirmed cases of urolithiasis. A significantly higher neutrophil counts (56.64%) in confirmed cases of urolithiasis compared to healthy and suspected groups (37.75 - 43.84%) and lower lymphocyte counts in confirmed cases indicated neutophilia and lymphocytopenia in animals that succumbed to death due to urolithiasis (Table 3).

Table 2. Ingredients used in lamb concentrate ration.

Ingredients	Percentage
Maize	50
Ground nut oil cake	15
Mustard	15
Wheat bran	18
Mineral mixture	1
Common salt	1

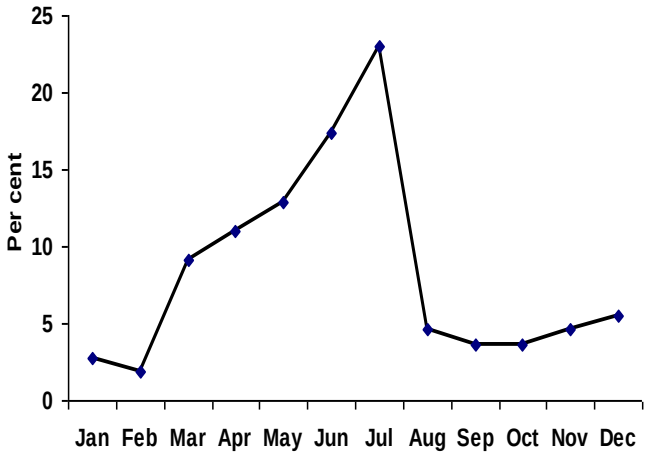

Figure 1. Monthly distribution of urolithiasis cases from 2002 – 2008.

Figure 2. Amorphous granular crystalline, irregular calculi of varying size in kidney.

Figure 3. Distended urinary bladder filled with urine.

Biochemical profile

Among various biochemical parameters studied, only levels of creatinine and serum urea nitrogen were found to be significantly elevated in confirmed cases of urolithiasis (7.05 ± 1.42 and 146.34 ± 24.87 mg/dl, respectively) compared to healthy group (Table 4). A moderate rise in levels of creatinine and serum urea nitrogen was also observed in suspected cases. The confirmed cases showed slight increase in total protein and albumin. The zinc containing enzyme alkaline phosphates was comparatively higher in confirmed cases of urolithiasis. The total cholesterol was within the normal range for the entire three groups.

Mineral profile

The mineral profile of concentrate showed there was imbalance of calcium, phosphorus ratio (Table 1). The mineral profile of blood exhibited that there was non-significant difference in values of Ca, P and Zn among different groups. However a significant rise in concentration of copper was noticed in suspected group and magnesium was noticed in suspected and confirmed groups (Table 5). The ration of Ca: P in blood varied from 1.34:1 (confirmed group) to 1.83:1.0 (suspected group) indicating disturbed ration of Ca and P. The confirmed cases of urolithiasis animals had slightly higher phosphorus and lowered calcium in serum. The magnesium content was significantly higher in suspected and confirmed cases, but in healthy animals it was within normal range.

Table 3. Mean ± SE haematological values in lambs affected with urolithiasis.

Parameter	Group			p-value
	Healthy	Suspected	Confirmed	
Hb (g/dl)	7.83 ± 0.50	7.92 ± 0.17	8.0 ± 0.17	0.887
PCV (%)	31.75 ± 2.17	32.92 ± 0.65	32.45 ± 0.78	0.749
TEC ($x10^6$)	6.43 ± 0.62	6.97 ± 0.16	6.64 ± 0.18	0.332
TLC ($x10^3$)	5.93 ± 0.14	6.04 ± 0.14	5.68 ± 0.19	0.275
Neutrophil (%)	37.75 ± 1.55^A	43.84 ± 2.93^A	56.64 ± 3.98^B	0.008
Lymphocyte (%)	60.75 ± 1.49^A	54.23 ± 2.69^A	40.90 ± 3.95^B	0.004
Eosinophil (%)	0.75 ± 0.48	1.0 ± 0.28	1.09 ± 0.25	0.823
Monocyte (%)	0.50 ± 0.29	0.54 ± 0.27	0.81 ± 0.23	0.679
Basophil (%)	0.25 ± 0.25	0.23 ± 0.12	0.54 ± 0.28	0.514

Means with different superscript differ significantly form each other in row.

Table 4. Mean±SE values for biochemical observations in lambs affected with urolithiasis.

Parameter	Group			p-value
	Healthy	Suspected	Confirmed	
Creatinine (mg/dl)	1.05 ± 0.23^A	1.78 ± 0.62^A	7.05 ± 1.42^B	0.001
Serum urea nitrogen ((mg/dl)	45.48 ± 8.37^A	67.38 ± 10.47^A	146.34 ± 24.87^B	0.004
Alkaline phosphatase(IU/lit)	182.57 ± 51.80	150.70± 26.30	232.77 ± 30.83	0.152
Cholesterol (mg/dl)	60.32 ± 6.84	56.06 ± 3.62	69.00 ± 6.24	0.191
Total protein (g/dl)	8.39 ± 1.22	7.76 ± 0.52	8.68 ± 0.81	0.626
Albumin (g/dl)	3.68 ± 0.87	3.66 ± 0.25	4.15 ± 0.25	0.470

Means with different superscript differ significantly from each other in row.

Table 5. Mean±SE concentration for serum minerals lambs affected with urolithiasis.

Parameter	Group			p-value
	Healthy	Suspected	Confirmed	
Ca (mg/dl)	5.39 ± 0.32	5.57 ± 0.24	5.03 ± 0.32	0.391
P (mg/dl)	3.21 ± 0.18	3.04 ± 0.18	3.76 ± 0.48	0.266
Ca:P	1.68:1.0	1.83:1.0	1.34:1.0	-
Zn (μg/dl)	3.89 ± 0.83	3.59 ± 0.34	3.90 ± 0.42	0.830
Cu (μg/dl)	1.57 ± 0.19^A	2.30 ± 0.12^B	1.85 ± 0.12^{AB}	0.009
Mg (mg/dl)	2.76 ± 0.26^B	5.00 ± 0.4^A	5.44 ± 0.30^A	0.018

Means with different superscript differ significantly from each other in row.

DISCUSSION

Urolithiasis occurs in all species but the problem assumes greatest economic importance in fattening livestock being fed heavy concentrated rations. The highest occurrence of urolith in male may be due to anatomical feature of urinary tract. Due to reduced ground water and rain, the grass cover in the summer months were very less and causes the lambs to eat sand along with grass, which may cause the imbalance in Ca: P ratio; in addition to this inadequate potable water predisposes the lambs to urolith formation. As the monsoon starts, the incidence of urolith problem started declining. Clinical signs associated with urolithiasis will depend upon the degree of blockage and severity of surrounding tissue reactions. If blockage is complete, retrograde pressure will build up in the bladder to the point of rupture and subsequent death of the animal. Incomplete blockage results in variable stages of stranguria, exaggerated and prolonged urination posture, urine dribbling and blood-tinged urine (Gerros,

1998). Affected animals may be depressed and lethargic, grind their teeth and show signs of abdominal distension and pain (Radostits et al., 2000). Rupture of the urinary bladder secondary to complete urethral obstruction by uroliths with leakage of urine into the abdominal cavity causes gradual accumulation of fluid from uroperitoneum resulting in a severely distended abdomen. Osmotic pressure from hypertonic urine together with acute fibrinous peritonitis promotes rapid movement of large amounts of extra cellular water into the peritoneal cavity resulting in abdominal distension and dehydration. Chemical peritonitis associated with uroperitoneum (Oehme and Tillmann, 1965) and cardiovascular disturbances caused by the abnormally high thoracic pressure induced by the abrupt release of large quantities of urine to the abdomen (Gasthuys et al., 1993) could also have contributed to some extent to the death of the animals. The presence of protein in urine might be due to renal damage, which may cause passage of large protein molecules into urine. The elevated serum urea and creatinine in suspected and confirmed lambs were due to rupture of bladder and uroperitoneum. These findings are in agreement with Sharma et al. (2005). Increase in serum urea nitrogen in clinically suspected animals is associated with starvation, infection, haemorrhage, pain and fever (Radostits et al., 2000). The elevated Hb, PCV, and TEC in confirmed cases may be due to haemo concentration and dehydration (Jadon et al., 1987; Singh et al., 1981). Similar trend was observed in clinically suspected cases. Sharma et al. (2005) noticed that animals with ruptured bladder had increased neutrophil count. Neutrophilia may be due to stress, which causes release of adrenaline that reduces stickiness of neutrophils with erythrocytes causing increase in neutrophil pool. Usually Neutrophilia was associated with uremia (Sastry, 1983). The confirmed cases showed slight increase in total protein level as compared to other two groups could be attributed to considerable loss of intra vascular water (Donecket and Bellamy, 1982). But Packett and Hauschild (1964) reported that serum potassium, sodium, total proteins, albumin and globulin had no apparent relationship with the development of urolithiasis. They also noted that abnormal phosphorus metabolism in development of ovine urolit-hiasis, increased levels of serum magnesium and phosphorus and lowered levels of calcium levels in ovine urolithiasis. Kunkel et al. (1953) found increased levels of serum magnesium in ovine urolithiasis and found the cause was due to changes in magnesium metabolism. Our results were also in a similar line. Crookshank and Robbins (1962) and Robbins et al. (1965) observed a reciprocal relation between the concentration of urinary magnesium and potassium and of urinary phosphorus and the in-cidence of calculi. In our study there were increased levels of serum magnesium and the cause may be due to changes in magnesium metabolism. The lamb concentrate ration of 50% maize might also be a con-tributory factor for calculi formation. Packett et al. (1959) and Udall (1959) had observed that the incidence of urolith was higher when the lambs were fed with high amount of maize in feed. Emerick et al. (1959) noted high serum phosphorus in lambs with urolithiasis although not receiving a high phosphorus diet. Emerick and Embry (1963) reported higher incidence of urinary calculi in lambs fed with higher phosphorus content in ration and ration with narrow Ca: P ratio. Stewart et al. (1993) found that the diets which higher silica content caused increased number of urolithiasis cases in rat model. Moreover, he also found that deficiency of either copper or zinc may contribute to urolithiasis with high intake of silica. Crookshank et al. (1967) found that there was a significant positive relationship between level of serum magnesium and proportion of lambs with urolithiasis. He also found that the animals, which developed urolithiasis, had lower calcium and higher phosphorus in serum when compared to non-urolith animals. In our study the variations in level of cholesterol, among different groups remained non-significant. The alkaline phosphates elevation in confirmed cases might be due to increased basal metabolic rate, excitement and pain. It is suggested that important contributing factors for the development of obstructive urolithiasis in that feedlot included rations with high levels of phosphorus, diminished water intake and dietary deficiency or lack of adequate amounts of fibre.

The formation of urinary calculi results when inorganic or organic urinary solutes are precipitated out of solution as crystals or amorphous deposits. The feeding of livestock on heavy concentrate-low roughage or pelleted rations results in increased production of mucoprotein which may act as a cementing agent favoring the formation of calculi (Radostits et al., 2000). Deficiencies in several macromolecules that inhibit crystallization of matrix in urine have also been suggested as a contributory factor to calculogenesis (Griffin, 1998; Atmani and Khan, 1999). Payne (1989) reported that the feedstuffs which contain phosphorus and magnesium in excess, but relatively low levels of calcium and potassium, predisposes to occurrence of the disease. There are many factors which increases the chance of urolithiasis, including heavy concentrate-low roughage diets, limited water intake, deprivation of water or dehydration, urine alkalinity, mineralized artesian water, alkaline water supplies, excess of sodium bicarbonate in the diet, vitamin imbalances e.g., hypovitaminosis A and hypervitaminosis D, and high-protein rations (Drolet and Dee, 1999; Radostits et al., 2000). During the summer season at semi-arid India there will not be any grass cover for grazing and the water availability will also be reduced. The suckling and weaner during that period will begin to consume sand which causes imbalance of calcium and phosphorus ratio in lambs leading to calculi formation. From the present study it was found that the

improper calcium-phosphorus ratio in feed, reduced water availability during summer predisposes the animals to urolithiasis. For preventive measures the chemical composition of uroliths together with environmental and dietary factors should be considered. Crystallization of urine can be prevented by dietary modification to induce urine acidification. The treatment of obstructive urolithiasis is primarily surgical but in large flock it may not be feasible. Critical preventive measures such as providing a Ca: P ratio of 2:1 in the complete ration, increasing the salt level to 4% of the diet in order to stimulate water consumption and to increase urine volume and the maintenance of adequate and abundant water supplies should be highly considered in feedlot management.

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