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Factors Promoting Silica Urolithiasis (Urinary Calculi) in Sheep

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FACTORS PROMOTING SILICA UROLITHIASIS (URINARY CALCULI) IN SHEEP[1]

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Summary

Sheep were used to study factors previously found to promote silica urolithiasis in a rat model. In addition to high silica, these dietary factors included elevated calcium, a high calcium to phosphorus ratio and alkali-forming effects. Wether lambs were fed ad libitum a diet of 50% grass hay and 50% ground oats plus supplement. Diet analysis was 3.4% SiO_2, .29% calcium, .25% phosphorus, 11.3% CP and 28% ADF. Treatments (40 lambs/ treatment) consisted of a control (C), limestone to increase dietary calcium to .6% (L), L + 1% sodium bicarbonate (LS) and L + 1% ammonium chloride (LA). After a 91-d experimental period followed by a 56-d post-experimental finishing period, silica kidney deposits were found in all treatments, and SiO_2 comprised 74% to 97% of the urolithic ash. Kidney urolith incidences in the four treatments were C, 7/40; L, 12/40; LS, 20/40; LA, 9/40. A higher urolith incidence in LS (LS vs C, $P<.05$), and a trend toward a higher incidence in L (L vs C, $P<.2$), accompanied by elevated urine pH (L = LS > C > LA, $P<.01$), lend support to the concept that high silica diets having high calcium to phosphorus ratios and alkali-forming potentials contribute to silica urolithiasis.

Introduction

Although the occurrence of silica uroliths in cattle has seemed inevitable in some studies simulating practical conditions (Whiting et al., 1958; Bezeau et al., 1961), attempts to produce silica uroliths experimentally in ruminants through the feeding of inorganic silicates under controlled conditions generally have been unsuccessful (Beeson et al., 1943; Emerick et al., 1959). Using a rat model (Emerick, 1984, 1986; Schreier and Emerick, 1986; Emerick and Lu, 1987) and in vitro studies (Emerick, 1987a), Emerick and co-workers identified dietary factors including low phosphorus concentrations, a high calcium to phosphorus ratio and alkali-forming effects as contributors to silica urolith formation in animals fed diets containing a high silica concentration. Additionally, lambs on drinking water treatments consisting of silicic acid equivalent to 1,500 and 3,000 mg SiO_2/liter showed a 9% to 45% incidence of silica kidney deposits (Emerick et al., 1988). Urinary silica concentrations did not differ between treatments, and the researchers concluded that the greater urine alkalinity and lower urinary phosphorus concentrations found to result from consumption of the silicic acid solutions were important contributors to silica urolith formation.

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The following study was conducted to determine the effects of a high calcium to phosphorus ratio and alkali-forming effects of the diet on silica urolithiasis in ruminants.

Materials and Methods

One hundred sixty wether lambs, averaging 61±15 lb. initially and of mixed Hampshire and Targhee breeding, were divided randomly from 10 weight groups into 16 pens of 10 lambs each. Each pen received only one lamb from each weight group. They were fed a basal diet (Table 1) of equal parts grass hay and oats plus supplement. The basal hay and concentrate diet contained .29% calcium and .26% phosphorus (Ca:P = 1.12). Treatments consisted of the basal diet (control, C),

TABLE 1. INGREDIENT AND CHEMICAL COMPOSITION OF DIETS

	Treatment			
Item	C[a]	L[b]	LS[c]	LA[d]
Ingredient, %				
Grass hay[e]	50	50	50	50
Oat grain[f]	49.7	48.9	47.9	47.9
Vegetable oil[g]	.08	.08	.08	.08
Trace mineralized salt	.2	.2	.2	.2
Lasalocid premix[h]	+	+	+	+
Vitamin A premix[i]	+	+	+	+
Limestone		.75	.75	.75
$NaHCO_3$			1.0	
NH_4Cl				1.0
Chemical composition[j], %				
DM	86.7	86.6	87.2	87.4
CP	11.5	11.3	11.3	13.0
ADF	28.2	28.0	27.5	27.6
Ca	.29	.59	.62	.57
P	.25	.26	.26	.24
SiO_2	3.38	3.39	3.40	3.42

[a]C (control treatment), Ca:P ratio was 1.12.
[b]L (limestone treatment), Ca:P ratio approximated 2.4 for all diets containing additional limestone.
[c]LS (limestone + sodium bicarbonate treatment), $NaHCO_3$ was included as 1% of the total diet.
[d]LA (limestone + ammonium chloride treatment), NH_4Cl was included as 1% of the total diet.
[e]Predominantly bromegrass.
[f]Soybean meal was added at a concentration of 2.25%, at the expense of oat grain, after 42 days of the experimental period.
[g]Vegetable oil was added for dust control during preparation of a premix.
[h]Lasalocid premix provided 20 mg lasalocid per kg of diet.
[i]Vitamin A premix provided 2,200 IU Vitamin A per kg of diet.
[j]Represents the average composition of diets (n = 7) during the 91-day experimental period.

.75% added limestone to increase diet calcium to .6% (L), L + 1% sodium bicarbonate (LS), and L + 1% ammonium chloride (LA). The hay, predominantly bromegrass, was ground (10 cm length) in a tub grinder and fed in equal quantity with oats plus supplement. The diets were fed once daily in amounts that would be almost completely consumed prior to the next feeding. Water was supplied ad libitum by automatic waterers.

After 42 days, the lambs had an average weight of 71±15 lb. Soybean meal,equivalent to 2.25% of the total diet, was added to all diets at the expense of oats in an attempt to increase growth rate of these small lambs. This had the effect of increasing dietary CP by 1 percentage unit while having a minimal effect on calcium or phosphorus concentrations.

Blood samples were collected from all lambs in heparinized tubes on day 42 of the experiment. Plasma was separated and stored at -20 °C. During a 2-week period starting on day 56 of the experimental period, 24-hour urine collections were obtained from one lamb per pen daily until urine had been obtained from all lambs. For urine collection, lambs were restrained in galvanized metabolism crates with access to diet and water. Urine was collected in plastic pails containing 25 ml toluene and filtered through multiple layers of cheese cloth. Urine pH was determined with a combination glass electrode, and an aliquot was stored at 2 °C for later analysis.

Calcium and magnesium concentrations in urine and plasma were determined by atomic absorption spectrophotometry[4] in the presence of .5% (wt/vol) lanthanum. Urine silica was measured by a silicomolybdate method as modified by Emerick (1984). Urine and plasma phosphorus were determined using the Fiske and Subbarow phosphomolybdate method (Oser, 1965).

At the end of the 91-day experimental period, the lambs averaged 82±16 lb. At that time, they were switched to a single high-concentrate finishing diet for an additional 56 days to achieve marketable weight. The finishing diet consisted of 90% rolled corn plus supplement and 10% ground alfalfa hay. By analysis, this diet contained .89% SiO_2, .71% calcium and .25% phosphorus (calcium to phosphorus ratio = 2.8) and would not be expected to contribute to urolith formation (Emerick and Embry, 1963).

At the end of the finishing period, the lambs averaging 114±20 lb. were slaughtered at an area packing plant. The kidneys were returned to the laboratory where they were opened and examined visually for the presence of uroliths. Uroliths were air dried, weighed, pooled by treatment and analyzed for silica, calcium, magnesium and phosphorus by methods described previously (Schreier and Emerick, 1986).

Statistical analysis of urolith incidence was by the chi-square method with treatments L, LS and LA being compared individually with treatment C (Steel and Torrie, 1980). Other data were analyzed by ANOVA using a model containing treatment and replication main effects (SAS, 1985). Treatment means were compared using the method of least significant difference protected by a significant F value (Steel and Torrie, 1980).

Results and Discussion

Four of the smallest lambs died during the first 6 weeks from heat stress and respiratory problems unrelated to the

[4]Perkin-Elmer atomic absorption spectrophotometer Model 503, Norwalk, CT 06856.

experiment. These included two from treatment L and 1 each from treatments LS and LA. None of these contained uroliths. One additional lamb from treatment C died of urinary obstruction during the post-experimental finishing period from a urolith lodged in the urethra. The incidence of uroliths, their weights and composition are shown in Table 2. With the one exception, these were recovered from the kidneys at slaughter.

Although the incidence of uroliths tended to be greater in all treatments that contained additional limestone (L, LS and LA) compared to the control (17.5%), only the LS treatment had significantly greater ($P<.01$) incidence (50%). This group also tended ($P<.2$) to have the greatest mass of urolithic material. Acid-forming ammonium chloride in treatment LA appeared to offer minimal protection from urolith formation (22.5% incidence for group LA vs 30% for group L, $P>.05$). However, in addition to the trend toward a lower urolith incidence, group LA also tended to have a lower mass of urolithic material that contained a lesser percentage of ash. For all treatments, silica was the principal inorganic urolithic component representing 73.9% to 96.7% of the ash.

Urinary characteristics and composition are shown in Table 3. Compared to treatment C, higher ($P<.05$) urine volumes occurred in treatments L and LS with an additional increase ($P<.05$) in treatment LA. Higher urine volume in sheep has been an inconsistent effect of feeding a variety of salts as 1% to 4% of the diet (Bushman et al., 1968), and a reduction in bovine silica urolith incidence from feeding sodium chloride at a rate approximating 4% of the diet has been attributed to increases in water consumption and urine volume (Bailey, 1981). The higher urine volume associated with the LS treatment was apparently not adequate to overcome the silica urolith-promoting effects of elevated dietary calcium and sodium bicarbonate.

Urinary pH values for treatments L and LS were higher ($P<.05$) than for the control (C) value. Sodium bicarbonate (LS) resulted in only a nominal increase over

TABLE 2. UROLITH INCIDENCE AND COMPOSITION

Item	Dietary treatments				SEM
	C[a]	L[b]	LS[c]	LA[d]	
Uroliths					
Incidence[e]	7/40[f]	12/40[f]	20/40[g]	9/40[f]	
Avg weight[h], mg	5.1	4.1	7.2	2.2	1.2
Ash, %[h,i]	70	80	86	53	
Ash composition, % of ash[i]					
SiO_2	96.0	96.7	94.8	73.9	
Calcium	3.3	.5	.9	2.3	
Magnesium	.3	.7	1.8	8.3	
Phosphorus	.7	.6	1.5	9.8	

[a,b,c,d]Same as Table 1.
[e]Number of sheep with uroliths/total number of sheep in treatment.
[f,g]Means in the same row without a common letter in their superscripts differ ($P<.05$).
[h]Air-dry basis.
[i]Uroliths were pooled by treatment.

TABLE 3. DEPENDENT VARIABLE MEANS INDICATING TREATMENT EFFECTS

Item	Dietary Treatments					
	C[a]	L[b]	LS[c]	LA[d]	SEM	P[e]
			Urinary characteristics[f]			
Volume, ml/d	513^{g}	786^{h}	801^{h}	$1,099^{i}$	66	.0001
pH	8.26^{g}	8.92^{h}	9.00^{h}	7.98^{i}	.09	.0001
			Urinary concentrations[f], mg/liter			
SiO_2	255	221	268	235	10	.06
Calcium	133^{g}	54^{h}	27^{h}	487^{i}	36	.0001
Magnesium	403^{g}	331^{h}	266^{h}	270^{h}	24	.0002
Phosphorus	37	22	9	9	11	.24
			Total urinary excretion[f], mg/d			
SiO_2	130^{g}	173^{h}	180^{h}	222^{i}	13	.0001
Calcium	52^{g}	38^{g}	20^{g}	412^{h}	16	.0001
Magnesium	169^{g}	224h	197^{gh}	239^{h}	15	.01
Phosphorus	20.2	19.1	7.4	7.5	7.6	.48
			Blood plasma concentrations, mg/dl			
Calcium	9.83^{g}	10.13^{h}	10.27^{hi}	10.45^{i}	.10	.0003
Magnesium	2.02	2.10	1.99	2.09	.03	.08
Phosphorus	6.54	6.59	6.96	6.75	.16	.30

[a,b,c,d]Same as Table 1.
[e]Probability value for treatment effect.
[f]Urine data are based on one 24-h collection from each lamb.
[g,h,i]Means in the same row without a common letter in their superscripts differ ($P<.05$).

that associated with limestone alone (L), possibly because urine rarely exceeds the approximate pH 9 observed in these treatments. On the other hand, ammonium chloride fed with limestone (LA) lowered ($P<.05$) urine pH .9 units below the value for limestone alone (L) and approximately .3 units below the value of the control (C).

With a 75% corn-25% alfalfa hay diet, Bushman et al. (1968) observed no effect of 2% calcium carbonate on urinary pH (pH = 8.7), and a decrease of only .4 pH units as a result of feeding 1% ammonium chloride. This suggests that lambs consuming the grass hay-oats diet in the current study produced urine that was less buffered in the range of pH 8 to 9 and more responsive to factors that promote changes in urine pH within this range. However, all treatments resulted in alkaline urine of a higher pH than was observed by Emerick and Lu (1987) to be associated with an ammonium chloride-suppression of silica uroliths in rats. These authors proposed a two-fold effect of urine acidification, i.e., urine acidification reduces the initial rate of polysilicic acid formation, and urinary phosphorus inhibits formation of polysilicic acid-protein complexes, the extent of the inhibition depending somewhat on pH. It appears that the reduction of urine pH by ammonium chloride in the current study may not have been adequate to achieve this effect. This result is in agreement with those reported by Bailey (1976) indicating that ammonium chloride did not prevent the formation of siliceous uroliths in calves.

Neither urinary silica nor phosphorus concentrations (Table 3) differed among treatments (P>.05). Compared to other treatments, urinary calcium concentrations were lower (P<.05) in treatments L and LS and higher (P<.05) in treatment LA. Urinary calcium concentrations were correlated negatively with urinary pH (r = -.56, P<.01). Total urinary excretion of calcium also was higher (P<.05) for treatment LA. The effect of ammonium chloride in increasing urinary calcium excretion is in agreement with previous reports from this laboratory (Bushman et al., 1967; 1968). No similar pH-related treatment effects on urinary magnesium concentrations were observed. Lower (P<.05) urinary magnesium concentrations in the L, LS and LA treatments, compared to the control, appeared to be due to the higher urine volumes of these groups because total urinary excretion of magnesium (Table 3) either did not differ from treatment C (P>.05, treatment LS) or was higher (P<.05, treatments L and LA).

Blood plasma calcium concentrations were lower (P<.05) for the C lambs than for the three other treatments. In addition to this effect attributable to limestone, a further increase (P<.05) was associated with ammonium chloride (treatment LA). Blood plasma concentrations of magnesium and phosphorus did not differ (P>.05) among treatments.

These data appear to be in agreement with the observations of Bezeau et al. (1961) who found uroliths, presumed to be silica, at birth in calves from cows producing alkaline urine on summer range and acidic urine while fed native prairie hay in winter. After 1 year on their study, calves from a group that produced alkaline urine throughout the year while supplemented with dried beet pulp and potassium bicarbonate had uroliths weighing more than four times those recovered from unsupplemented calves.

Data reported here lend support to the concept, developed with a rat model (Emerick, 1984, 1986; Schreier and Emerick, 1986; Emerick and Lu, 1987), that diets having a high calcium to phosphorus ratio and an alkali-forming potential contribute to silica urolithiasis. However, a simple relationship between urine pH and silica urolithiasis in ruminants has not been established, and the addition of ammonium chloride alone to sheep diets containing elevated calcium was ineffective in preventing silica urolith formation. For minimizing silica urolith formation, the recommendation (Emerick, 1987b) that animals grazing range grass be supplemented with a phosphorus source having an acid-forming potential appears plausible.

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