EFFECTS OF DIETARY AMMONIUM CHLORIDE AND VARIATIONS IN CALCIUM TO PHOSPHORUS RATIO ON SILICA UROLITHIASIS IN SHEEP[1,2]

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ABSTRACT

Ammonium chloride was added to diets varying in Ca content to evaluate its potential in preventing silica urolith formation in sheep. A 2 × 2 factorial experiment involved wether lambs with ad libitum access to a diet of 50% grass hay and 50% ground oats plus supplement. The basal diet contained on a DM basis 3.3% SiO_2, .31% Ca, .22% P, 11.6% CP, and 26% ADF. Treatments (38 to 39 lambs/treatment) consisted of a control (C), limestone to increase dietary calcium to .6% (L), 1% ammonium chloride (A), and L + A (LA). After a 118-d experimental period, siliceous kidney deposits were found only in C and L, with silica making up 93% to 95% of the urolithic ash. Urolith incidences were 13% (C) and 18% (L), respectively. The lack of urolith development in lambs fed A and LA (ammonium chloride effect, $P < .01$) and a trend toward a lower urolith incidence in C vs L ($P < .2$) support the hypothesis that acid-forming effects of the diet and a reduction in the dietary Ca to P ratio reduce silica urolith formation.

Key Words: Sheep, Silica, Urolithiasis, Ammonium Chloride, Calcium, Phosphorus

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Introduction

Silica urolithiasis is considered to be a major nutritional disorder mainly afflicting grazing ruminants under range conditions. High urinary alkalinity and low urinary P content, prompted by a high dietary Ca to P ratio, have been identified as major contributors to siliceous urolith formation in rats (Emerick, 1984, 1986; Schreier and Emerick, 1986; Emerick and Lu, 1987) and in sheep (Emerick et al., 1988; Stewart et al., 1990). These researchers found that supplemental P reduced the incidence of silica uroliths in rats and sheep. Although supplemental ammonium chloride reduced incidence of silica uroliths in rats, it seemed to be ineffective in sheep when added to a diet containing an elevated level of Ca (Stewart et al., 1990).

This study was conducted to evaluate the effectiveness of ammonium chloride for reducing silica urolith formation in sheep when added to diets having either a high (2:1) or low (1:1) Ca to P ratio resulting from variations in dietary Ca.

Materials and Methods

One hundred fifty-six crossbred wether lambs of predominately Hampshire and Targhee breeding, initially averaging 32.8 ± 3.6 kg BW, were placed into 13 weight groups from which they were randomly allotted into 12 pens of 13 lambs each. Each pen received only one lamb from each weight group. The basal diet (Table 1), composed to provide a high concentration of silica, consisted of equal parts grass hay and oats plus supplement. It contained .99 Mcal NE_g/kg (calculated DM basis), and on an as-fed basis it contained 3.24% SiO_2, .31% Ca, and .22% P (Ca:P = 1.4). Four treatments consisted of the basal diet (control, C), .75% added limestone to

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TABLE 1. INGREDIENT AND CHEMICAL COMPOSITION OF DIETS

Item	Treatment			
	C[a]	L[b]	A[c]	LA[d]
Ingredient, %				
Grass hay[e]	50	50	50	50
Oat grain	44.5	43.8	43.5	42.8
Soybean meal (44% CP)	2.2	2.2	2.2	2.2
Animal fat	3.0	3.0	3.0	3.0
Trace mineral salt[f]	.2	.2	.2	.2
Lasalocid premix[g]	+	+	+	+
Vitamin A premix[h]	+	+	+	+
Limestone	—	.75	—	.75
NH_4Cl	—	—	1.0	1.0
Chemical composition[i], %				
DM	91.4	91.3	91.6	91.3
CP	11.6	11.4	12.8	12.8
ADF	26.2	27.4	27.3	26.6
Ca	.31	.63	.31	.61
P	.22	.23	.21	.23
SiO_2	3.24	3.19	3.34	3.29

[a]C = control treatment; Ca:P ratio was 1.4.

[b]L = limestone treatment; limestone was added as .75% of the total diet; Ca:P ratio approximated 2.7 for all diets containing additional limestone.

[c]A = ammonium chloride treatment; NH_4Cl was included as 1% of the total diet.

[d]LA = limestone + ammonium chloride treatment.

[e]Predominantly mature *Bromus* species.

[f]Morton salt, division of Morton Thiokol, Inc., Chicago. IL. The trace mineralized salt contained the following: NaCl, 93 to 98%; Zn (ZnO), .350%; Mn (MnO), .280%; Fe (Fe carbonate), .175%; Cu (CuO), .035%; I (Ca periodate), .007%; Co (Co carbonate), .007%.

[g]Lasalocid premix provided 20 mg lasalocid per kilogram of diet.

[h]Vitamin A premix provided 2,200 IU Vitamin A per kilogram of diet.

[i]Represents the average composition of diets (n = 8) during the 118-d experimental period.

increase dietary Ca to .6% (L), 1% added ammonium chloride (A), and L + A (LA). Treatments were replicated with three pens per treatment. The hay, predominantly mature *Bromus* species, was ground (10-cm length) in a tub grinder before feeding. The diets were fed once daily in amounts that would be almost completely consumed before the next feeding. Water supplied ad libitum by automatic waterers was from the same source as that used by Stewart et al. (1990). The water source was Brookings city water containing approximately 800 mg total dissolved solids/liter, including 175 ppm Ca and 50 ppm Mg; the principal anions were sulfate and bicarbonate.

Blood samples were collected from all lambs by jugular venipuncture in heparinized tubes before feeding on d 41, and plasma was separated and stored at –20°C. During a 9-d period starting on d 62, a 24-h urine collection was obtained from each lamb. For urine collection, lambs were restrained in galvanized metabolism crates with access to diet and water. Urine was collected in plastic pails containing 30 ml of toluene and was filtered through multiple layers of cheesecloth. Urinary pH was determined with a combination glass electrode.

Following determination of urine silica by a silicomolybdate method as modified by Emerick (1984), an aliquot of urine was acidified with HCl and stored at 2°C for further analyses. Calcium and Mg concentrations in urine and plasma were determined by flame atomic absorption spectrophotometry[6] in the presence of .5% (wt/vol) lanthanum using instrument parameters recommended by the equipment manufacturer. Urine and plasma P were determined using the Fiske and Sub-

[6]Model 503, Perkin-Elmer, Norwalk, CT.

TABLE 2. UROLITH INCIDENCE AND COMPOSITION

Item	Dietary treatments				
	C[a]	L[b]	A[c]	LA[d]	SEM
Uroliths					
Incidence[e]	5/38[f]	7/39[f]	0/38[g]	0/39[g]	
Avg weight[h], mg	3.7	5.4	—	—	1.9
Ash, %[hi]	73	62	—	—	
Ash composition, % of ash[i]					
SiO_2	95.2	93.7	—	—	
Ca	.83	.73	—	—	
Mg	1.46	.89	—	—	
P	1.1	.81	—	—	

[a]C = control treatment; Ca:P ratio was 1.4.

[b]L = limestone treatment; limestone was added as .75% of the total diet; Ca:P ratio approximated 2.7 for all diets containing additional limestone.

[c]A = ammonium chloride treatment; NH_4Cl was included as 1% of the total diet.

[d]LA = limestone + ammonium chloride treatment.

[e]Number of sheep with uroliths/total number of sheep in treatment.

[f,g]Data with superscripts that do not have a common superscript letter differ ($P < .01$).

[h]Air-dry basis.

[i]Uroliths were pooled by treatment.

barow phosphomolybdate method (Oser, 1965).

At the end of the 118-d experimental period, the lambs averaged 45.6 ± 5.7 kg BW. At that time, they 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, Ca, Mg, and P by methods described previously (Schreier and Emerick, 1986).

Statistical analysis of urolith incidence was by the chi-square method; treatments L, A, and LA were compared individually with treatment C (Steel and Torrie, 1980). Other data were analyzed by GLM procedure using a model consisting of limestone, ammonium chloride, and replications as main effects and all two- and three-way interactions (SAS, 1985). Main effects and interactions were tested against the residual mean squares. Differences ($P < .05$) between appropriate means were identified by the method of lsd protected by a significant F-value (Steel and Torrie, 1980).

Results and Discussion

Two lambs, one each in treatments C and A, died of pneumonia within the first 2 wk of the experimental period and were deleted from the experiment. Average DMI (kg/d) were 1.11 (C), 1.10 (L), .99 (A), and 1.04 (LA) (ammonium chloride effect, $P < .01$). The ADG across all treatments was .109 kg/d, with no difference (SEM = .005, $P > .05$) among treatments.

The incidence of uroliths and their weights and composition are listed in Table 2. Uroliths occurred only in treatments devoid of ammonium chloride (treatments C and L). The incidence was 13% in treatment C, 18% in treatment L, and 0 in treatments A and LA. Silica was the principal inorganic urolithic component (Table 2), representing 95% (C) and 94% (L) of the ash. These data, indicating that ammonium chloride in treatments A and LA offered protection from silica urolith formation, are more positive than those reported previously (Stewart et al., 1990). There seemed to be a lower urolith incidence in groups C and L than was observed for comparable groups in the previous study; this may indicate that conditions promoting urolith formation in this study were less severe and, therefore, more readily nullified by ammonium chloride. The less severe urolithic conditions in this study seem to have included higher urine volumes with correspondingly lower urinary silica concentrations and(or) lower urinary pH values.

Urinary characteristics and composition are shown in Table 3. Urine volume was higher (P

TABLE 3. EFFECT OF DIETARY AMMONIUM CHLORIDE AND LIMESTONE ON URINE VOLUME, pH, MINERAL CONCENTRATIONS, AND PLASMA MINERAL CONCENTRATIONS

	Dietary treatments					
Item	C[a]	L[b]	A[c]	LA[d]	SEM	P[e]
Urinary characteristics[f]						
Volume, ml/d	824[g]	896[g]	1,373[h]	947[g]	90.8	.007
pH	8.55[g]	8.61[g]	7.74[h]	7.77[h]	.08	.0001
Urinary concentrations[f], mg/liter						
SiO_2	212[g]	196[g,h]	188[h]	233[i]	8.4	.02
Ca	101[g]	66[g]	230[h]	322[i]	34	.0001
Mg	519	499	379	506	32	.20
P	17	11	44	26	13.9	.34
Total urinary excretion[f], mg/d						
SiO_2	169[g]	167[g]	283[h]	200[i]	13.1	.02
Ca	79[g]	54[g]	296[h]	243[i]	25.4	.0001
Mg	379	384	521	416	40.2	.26
P	11.2	8.9	39.7	20.4	10.9	.27
Blood plasma concentrations, mg/dl						
Ca	9.93	10.24	10.10	9.99	.11	.60
Mg	1.98	2.01	1.99	1.95	.02	.36
P	6.20	6.12	6.39	6.22	.09	.18

[a]C = control treatment; Ca:P ratio was 1.4.

[b]L = limestone treatment; limestone was added as .75% of the total diet; Ca:P ratio approximated 2.7 for all diets containing additional limestone.

[c]A = ammonium chloride treatment; NH_4Cl was included as 1% of the total diet.

[d]LA = limestone + ammonium chloride treatment.

[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 ($P < .05$).

< .01) for treatment A than for all other treatments. Urine pH, approximately 8.6 in treatments C and L, was reduced ($P < .01$) an average of .82 pH units by the 1% dietary concentration of ammonium chloride in treatments A and LA. Bailey (1981) reported that an increase in water consumption, and presumably an increase in urine volume, prompted by the feeding of sodium chloride reduced the incidence of silica uroliths. An increase in urine volume from the feeding of ammonium chloride in treatment A may have contributed to the reduction in silica urolith formation in that group. However, a similar effect of ammonium chloride on urine volume was not observed in treatment LA. Reduction in urine pH was a more consistent effect of the ammonium chloride common to both A and LA treatments.

Compared with the control, dietary limestone had no effect ($P > .05$) on urinary volume or pH. Further, limestone, in the absence of ammonium chloride, had no effect (L vs C, $P < .05$) on the urinary concentration or total daily urinary excretion of silica, Ca, Mg or P. Ammonium chloride most notably increased (A vs C, $P < .05$) urinary Ca and silica (mg/d) excretion. Total daily urinary Ca and silica excretions were subsequently lowered (LA vs A, $P < .05$) by limestone. However, a higher urinary volume in treatment A and the associated greater dilution of these constituents resulted in lower ($P < .05$) urinary concentrations of Ca and silica in treatment A than in treatment LA. A negative correlation (r = −.59, $P < .01$) existed between urinary pH and urinary Ca concentrations in this study. The effect of ammonium chloride in increasing urinary Ca excretion in sheep is in agreement with previous work from this laboratory (Bushman et al., 1967, 1968; Stewart et al., 1990).

No treatment effects ($P > .05$) were observed on blood plasma concentrations of Ca, Mg or P. This is contrary to the report by Stewart et al. (1990) that limestone and ammonium chloride individually increased plasma Ca.

These data showing a siliceous urolith prophylactic effect for ammonium chloride are in contrast to the findings of Bailey (1976) that 30 g of ammonium chloride administered daily to steers fed range-grass hay did not influence urinary silica deposition. However, 30 g per steer represented a lower quantity of ammonium chloride proportionate to BW than the approximately 12 g per lamb consumed in this study. On the other hand, the data are consistent with those of Stewart et al. (1990), which indicated a trend toward lower silica deposition in sheep fed a grass-hay, oat-grain diet containing 1% ammonium chloride.

Emerick and Lu (1987) found that ammonium chloride suppressed silica urolithiasis in rats, and they postulated that factors contributing to urine acidification tend to reduce the initial rate of polysilicic acid formation and facilitate an inhibitory effect of P on the formation of polysilicic acid-protein complexes. The formation of polysilicic acid-protein complexes, generally insoluble, may represent the mechanism for formation of silica uroliths (Bailey, 1972).

A reduction in urinary pH is the only effect of ammonium chloride that is consistent with factors that have been demonstrated to reduce urinary silica deposition. Although increases in dietary Ca have been shown to contribute to formation of silica uroliths in rats (Schreier and Emerick, 1986), variations in dietary Ca concentrations in this study had no effect on the efficacy of ammonium chloride for reducing silica urolith formation.

In this study the urolith incidence tended to be less than that observed by Stewart et al. (1990) for lambs on similar treatments, and it was severalfold less than the 100% incidence in steers that failed to benefit from ammonium chloride in the study by Bailey (1976). The variable effects reported for ammonium chloride on silica urolith formation in ruminants may be related to differences in severity of the urolithic conditions existing in the various studies.

Implications

This study lends additional strength to the concept that an increase in the acid-forming potential of the diet is an important condition for minimizing silica urinary deposits.

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