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Effects of dietary aluminum source and concentration on mineral status of feeder lambs

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ABSTRACT

A 100 d experiment was conducted to determine the effects of aluminum (Al) source and concentration on mineral status, emphasizing phosphorus (P), of 50 feeder lambs. Six treatments, fed at 10% of the total diet, were formulated using two sources of Al, $AlCl_3$ and an Al-based water treatment residual (WTR, 11.1% Al), with varying levels of Al and P: (1) control (10% sand, C), (2) low WTR (2.5% WTR and 7.5% sand, L-WTR), (3) $AlCl_3$ with added P (1% $AlCl_3$, 9% sand, and 0.4% P, $AlCl_3$ + P), (4) high WTR (10% WTR, H-WTR), (5) $AlCl_3$ (1% $AlCl_3$ and 9% sand, $AlCl_3$), and (6) high WTR with added P (10% WTR and 0.4% P, H-WTR + P). The total Al varied from 0.037 to 1.2% among diets. Only lambs fed the high WTR diet without P supplementation (H-WTR) decreased feed intakes. These lambs consumed about half as much feed as lambs on all the other treatments, and had lower ($P < 0.05$) BW from d 84 on. Lambs receiving the H-WTR had the lowest bone Ca, P and Mg concentrations (fresh basis, mg/cm^3) and lowest bone mineral content (BMC) as determined by radiographs (mm of Al). Results for the lambs on H-WTR were confounded by the greatly reduced feed intake of animals on this treatment. Plasma P decreased in all lambs consuming Al, regardless of Al source, but the effects were less severe in animals provided additional P supplementation ($AlCl_3$ + P and H-WTR + P). Apparent absorption of P was affected by concentration and source of Al in two metabolism trials ($n = 42$) beginning on d 34 and d 70, respectively. In the first trial, d 34, lambs receiving $AlCl_3$ treatment had reduced apparent P absorption, −17.7% ($P < 0.05$), when compared to all other treatments. In the d 70 trial, lambs receiving both $AlCl_3$ and H-WTR treatments were negatively impacted ($P < 0.05$) compared to the control, −20.9 and −2.5% apparent P absorption, respectively, but were no longer different from one another ($P > 0.05$). Diets containing 1.2% Al as WTR without P supplementation depressed feed intakes, weight gains, plasma P concentrations ($P < 0.05$), and BMC. However, given adequate P supplementation, even lambs consuming this amount of Al did not suffer detrimental effects, as lambs on H-WTR + P did not differ from the control ($P > 0.05$) in feed intakes, weight gains, or BMC.

1. Introduction

Of the issues facing environmentalists worldwide, water pollution is at the forefront. Phosphorus (P) is one of the leading nutrients in contaminated water ways and is generally the main freshwater problem (Parry, 1998). Much P

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pollution stems from agricultural drainage and from the runoff and leaching of various wastes (Sims et al., 1998). Soil amendments, like aluminum (Al), decrease P leaching by increasing a soil's capacity to retain P (Elliott et al., 2002; Dayton et al., 2003). Aluminum chloride ($AlCl_3$) is one such amendment; however, it is a highly bioavailable form of Al that may result in toxicity, observed as P deficiency, if ingested by livestock (Valdivia, 1977). Dietary Al can suppress sheep voluntary feed intake, feed efficiency, plasma P, and weight gains (Rosa et al., 1982). Additional dietary P decreases, but does not eliminate, the negative effects of Al.

Water treatment residuals, by-products from some drinking water treatment processes, can be another soil amendment choice. While Ca, Fe or Al can be used as the primary mineral to remove impurities from the drinking water, this study used an Al-based water treatment residual (WTR), previously shown to immobilize P (Makris et al., 2005). Grazing animals may consume as much as 10–15% of their dry matter intake (DM) as soil, depending on soil and pasture conditions (Field and Purves, 1964; Healy, 1968). The question arises then as to whether animals would consume sufficient quantities of WTR to be detrimental. Unlike $AlCl_3$, the bioavailability of Al in WTR is expected to be low (O'Connor et al., 2002). Previous studies have shown that WTR was not detrimental to animals when P levels are above adequate (Madison, 2007; Van Alstyne et al., 2007). The study by Van Alstyne et al. (2007) showed that WTR when consumed as 10% DM intake (0.80% Al and 0.25% P) was not detrimental to sheep.

The following experiment was carried out to evaluate a WTR with a higher Al concentration and to have a diet with a lower level of P, comparable to many pastures low in P, which did not exceed sheep requirements. The purpose of this study was to determine if the effects of Al as WTR would be less detrimental to animal growth, feed intake, plasma P levels, bone mineral content (BMC), and apparent P absorption then Al as $AlCl_3$.

2. Materials and methods

2.1. Animals and management

Fifty 5 to 8 month old Dorper × Katahdin lambs (41 rams and 9 wethers) were used in a 100 d trial at the University of Florida Sheep Unit located in Gainesville, Florida. Lambs were produced in Tennessee and shipped to Gainesville. The lambs weighed 13.2–41.8 kg on d 0. Weight disparity was caused by lambs being on pasture prior to shipment. The trial ran from 24 October 2006 to 1 February 2007.

Lambs were treated for health concerns prior to d 0. On 25 September 2006, animals were given clostridium vaccination, tetanus toxoid and ivermectin (Ivomec; Merial Ltd., Iselin, NJ). Animals received Dectomax® (Pfizer Animal Health, Exton, PA) and Corid® (Corid 9.6%; Merial, Duluth, GA) on 4 October 2006 to treat *Haemonchus contortus* worms and coccidiosis, respectively. Twelve sheep were also treated for mild infections with oxytetracycline (Liquamycin LA-200; Pfizer Animal Health, Exton, PA) with consecutive treatments on 8 and 11 October. Animal 141 received a blood transfusion on 10 October 2006. Two treatments of Cydectin® (Fort Dodge Animal Health, Overland, KS) were given on 18 October and 5 November for maintenance parasite control to all lambs.

A corn based basal diet was formulated to meet NRC (1985) requirements for CP, TDN, vitamins and minerals for growing lambs. The basal diet was formulated to contain 0.17% P on a DM basis. This borderline to low P concentration was used to better elucidate the effect of dietary Al on P.

Lambs were randomly assigned to one of six dietary treatments and were housed with either three to five animals per pen in covered pens (24 m^2) with earthen floors. Prior to the start of the experiment, all animals were fed the control diet containing 10% sand at 0.45 kg per animal per day (d). After week 1 feed was increased to 1.1 kg per animal per d to optimize growth. During the trial, lambs were fed once daily 1.1 kg per animal per d (as-fed) and were given access to *ad libitum* water. The dietary treatments (Table 1) were added at 10% of the total diet fed, as follows: (1) control (10% sand, C), (2) low WTR (2.5% WTR and 7.5% sand, L-WTR), (3) $AlCl_3$ with added P (1% $AlCl_3$, 9% sand, and 0.4% P, $AlCl_3$ + P), (4) high WTR (10% WTR, H-WTR), (5) $AlCl_3$ (1% $AlCl_3$ and 9% sand, $AlCl_3$), and (6) high WTR with added P (10% WTR and 0.4% P, H-WTR + P). The WTR contained 11.1% Al, 0.38% Fe, and 0.28% P on a dry basis. The sand contained 0.1% Al, 0.026% Fe, and 0.002% P on a dry basis. Thus, the total Al concentrations of the diets were 0.037 (C), 0.30 (L-WTR), 0.31 ($AlCl_3$ + P), 1.2 (H-WTR), 0.31 ($AlCl_3$), and 1.2% (H-WTR + P) on a DM basis. The protocol for this study was approved by the University of Florida Institutional Animal Care and Use Committee (E690).

2.2. Sample collection and analysis

Weights and blood samples were obtained for each animal on d 0, 28, 56, 84, and 98. Blood samples were collected using a vacutainer system (Vacutainer; Becton Dickinson, Franklin Lake, NJ) into tubes containing sodium heparin as an anticoagulant. Samples were centrifuged at 2147 × *g* for 30 min. The collected plasma was frozen at −21 °C until later analysis. One mL of thawed plasma was deproteinated with 9 mL of 10% trichloroacetic acid and then analyzed for Al, Ca, Cu, Fe, Mg, P, and Zn (Miles et al., 2001).

Dorsospalmar radiographs were obtained of the left third metacarpal on d 0, 48, and 98 via a portable x-ray machine (Easymatic Super 325; Universal X-Ray Products, Chicago, IL) at a focal length of 91.5 cm and an exposure of 97 kVp (30 mA for 0.067 s). An 11-step wedge was taped to the radiograph cassette next to the leg and simultaneously exposed as a reference standard for the radiograph. The films were exposed with an auto-radiograph processing machine using Kodak products and development procedures (Eastman Kodak Co., Rochester, NY). Optical density was assessed with an imaging densitometer and software that translated the digital image to numeric values by scanning medial to lateral 1 cm below the nutrient foramen (Image-Pro Plus, Media Cybernetics, Inc., Silver Springs, MD). A linear regression of the optical density of the bone (expressed in mm of Al) was plotted using the known thickness of the steps on the Al step wedge (Van Alstyne et al., 2006).

Apparent P and Al absorptions were determined on 42 lambs fitted with cloth fecal collection harnesses and placed in metabolism crates (1.4 m^2) for two collection periods starting on d 34 and d 70, respectively. Water was offered *ad libitum* and lambs were fed 1.1 kg/d and orts were collected daily. No marker was used as animals had been fed same diets prior to the collection period. Lambs were given a 3 d adaptation period followed by 7 d of collection. Ten percent of each collection was saved and composited for DM, P and Al analysis (Miles et al., 2001).

Animals were slaughtered on d 100 at a USDA-inspected facility. Tissues (liver, kidney, heart, and muscle) were collected for analysis of Al, Cu, Fe, Mn, P, and Zn. Brain was also collected and analyzed for Al. The left metacarpal was collected for analysis of Al, Ca, Mg, and P (Miles et al., 2001). Bones were skinned and wrapped in cheesecloth (preciously soaked in 0.9% saline solution) and frozen at 0 °C until analysis. After thawing, a 2 cm section of bone was cut to include the section of scanned bone 1 cm below the nutrient foramen. Marrow was removed and bones were rinsed in saline solution and placed on clean cheesecloth. Density was determined and expressed as g/cm^3, fresh basis (Kit ME-40290, Mettler Instruments Corp., Hightstown, NJ) (Van Alstyne et al., 2006).

For all samples, P was analyzed via the colorimetric procedure (Harris and Popat, 1954) on a microplate reader (KC junior software; BioTek® Instruments Inc., Winooski, VT). In all samples except the plasma and bone, Al concentrations were analyzed via atomic absorption spectrophotometry using nitrous oxide-acetylene flame (PerkinElmer Model Analyst 800, PerkinElmer Corp., Norwalk, CT). Blood and bone Al concentrations were analyzed by inductively coupled plasma-atomic emissions spectroscopy, a more sensitive analysis (ICP-AES) (PerkinElmer Plasma 3200, PerkinElmer, Wellesley, MA). All other minerals in all tissues, feces, and feed were analyzed via flame atomic absorption spectrometry (PerkinElmer Model Analyst 800, PerkinElmer Corp., Norwalk, CT). To ensure quality of data and analytical methods, standards were prepared simultaneously with

Table 1
Diet composition (as-fed) and mineral analyses of treatments

Ingredient (%, as fed)	Treatments[a]					
	1	2	3	4	5	6
Ground corn	27.9	27.9	27.5	27.9	27.9	27.5
Cottonseed hulls	18.9	18.9	18.6	18.9	18.9	18.6
Corn starch	25.1	25.1	24.8	25.1	25.1	24.8
Molasses[b]	3.6	3.6	3.55	3.6	3.6	3.55
Soybean meal	2.7	2.7	2.66	2.7	2.7	2.66
Corn oil	3.6	3.6	3.55	3.6	3.6	3.55
Alfalfa meal	2.7	2.7	2.66	2.7	2.7	2.66
Sand[c]	10	7.5	9	–	9	–
Water treatment residual[d]	–	2.5	–	10	–	10
Aluminum chloride	–	–	1	–	1	–
Dicalcium phosphate	–	–	1.3	–	–	1.3
Blood meal	1.8	1.8	1.77	1.8	1.8	1.77
Urea	1.35	1.35	1.33	1.35	1.35	1.33
Mineral–vitamin premix[e]	0.95	0.95	0.94	0.95	0.95	0.94
Limestone	0.9	0.9	0.89	0.9	0.9	0.89
Ammonium chloride	0.45	0.45	0.44	0.45	0.45	0.44
Chloratetracycline	0.045	0.045	0.044	0.045	0.045	0.044
Analyses[f]						
Ca (%)	0.62	0.63	0.9	0.71	0.59	1.00
K (%)	0.50	0.54	0.49	0.58	0.52	0.55
Mg (%)	0.092	0.096	0.098	0.100	0.092	0.100
Na (%)	0.42	0.39	0.38	0.41	0.37	0.39
P (%)	0.18	0.18	0.34	0.19	0.14	0.34
Al (%)	0.037	0.300	0.310	1.200	0.310	1.200
Cu (mg/kg)	7.2	14.3	17.4	27.2	11.2	28.5
Fe (mg/kg)	535	356	349	528	217	579
Mn (mg/kg)	41.3	39.1	35.1	35.3	34.2	34.9
Zn (mg/kg)	73.9	83.8	87.1	98.5	82.4	94.1

[a] Treatments are as follows: (1) control ($n=9$), (2) low WTR ($n=8$), (3) $AlCl_3$ + P ($n=7$), (4) high WTR ($n=9$), (5) $AlCl_3$ ($n=9$), and (6) high WTR + P ($n=8$).
[b] Suga-Lik™ 16% slurry with 5% Catfish oil: 74% DM, 16% CP, 5% CF, and 35% total sugar.
[c] Sand contained 0.1% Al, 0.026% Fe, and 0.002% P.
[d] Water treatment residual contained 11.2% Al, 0.38% Fe, and 0.28% P.
[e] Contained 1 ppm Co (as carbonate), 5 ppm Cu (as oxide), 0.7 ppm I (as iodate), 35 ppm Fe (as carbonate and oxide), 25 ppm Mn (as oxide), 0.2 ppm Se (as sodium selenite), 0.2 ppm S (as flowers of sulfur), 75 ppm Zn (as oxide), vitamin A at 5000 IU/kg, vitamin D at 500 IU/kg, and Vitamin E at 15 IU/kg.
[f] Dry matter basis: as % of the diet or mg of element/kg of diet.

certified National Bureau of Standards (NBS) materials (citrus leaves SRM-1572; Bovine liver SRM-1577a; bone ash SRM-1400), acquired from the National Institute of Standards and Technology (NIST; Gaithersburg, MD). For a given sample run, if the NBS standards resulted in values outside the acceptable range for that reference material, data for that element was not accepted; the instrument was recalibrated and the analysis run again. Calibration standard curves were recalibrated every 25 samples with quality control (QC) checks performed to ensure the precision of the instrument. Spiked recoveries were within 10%.

2.3. Statistical analysis

The experiment was a completely randomized design. Plasma, BW, and radiograph data were analyzed as a factorial with repeated measures over time and a variance component with respect to time using PROC MIXED in SAS (SAS for Windows v8.1; SAS Inst. Inc., Cary, NC). Post hoc testing was done. The alpha level used was 0.05 with Bonferroni adjustments for multiple comparisons when necessary.

3. Results and discussion

Lambs receiving H-WTR had lower ($P<0.05$) feed intakes beginning on week 6. This is likely due, in part, to the fact that lambs were fed individually for the first time when placed in metabolism crates from week 4 to week 6. Animals may have suffered some separation anxiety as an average decrease in feed intake occurred with all lambs, not just those receiving Al. Likewise, during the second time sheep were placed in metabolism crates (week 10 to week 12), feed intake decreased, although not as severely.

Body weights increased in all treatments from d 0 to d 98 (Table 2). Lambs receiving H-WTR had lower body weights ($P<0.05$) then lambs on C, $AlCl_3$ + P, and $AlCl_3$ on d 84 and lower body weights than those on all treatments except the L-WTR on d 96 ($P<0.05$). The results are likely due to the fact that animals in the H-WTR treatment

Table 2
Effects of dietary Al and P on body weight of feeder lambs (kg)[a]

Day	Treatment[b]					
	1	2	3	4	5	6
0	28.8	25.8	28.9	27.0	28.6	27.0
28	32.2	29.5	33.7	27.3	31.5	31.3
56	36.1	31.8	36.2	28.4	34.7	33.9
84	39.9 a	35.0 ab	40.2 a	28.6 b	38.8 a	36.8 ab
98	42.2 a	36.7 ab	41.1 a	29.2 b	40.4 a	37.8 a
S.E.	2.21	2.34	2.50	2.21	2.21	2.34

[a] Means within rows lacking common letters (a,b) differ ($P<0.05$); adjusted for multiple comparisons. The S.E. for day effect is 0.941.
[b] Treatments are as follows: (1) control ($n=9$), (2) low WTR ($n=8$), (3) $AlCl_3$ + P ($n=7$), (4) high WTR ($n=9$), (5) $AlCl_3$ ($n=9$), and (6) high WTR + P ($n=8$).

Table 3
Effect of dietary Al and P on feed intake (g/lamb/d)[a,b,c]

Week	Treatment[d]					
	1	2	3	4[e]	5	6
4	1130	1130	1120	1070	1120	1090
6	1090	1110	1100	626	1090	1060
8	1130	1120	1110	885	1120	1130
10	1130	1130	1100	736	1130	1130
12	1130	1120	1070	682	1120	1120
14	1130	1130	1130	625	1130	1130

[a] Feed was increased after 1 week on trial to allow for optimum growth. The S.E. for week effect is 74.20.

[b] Al intakes (g/(lamb d)): C = 0.41, L-WTR = 3.4, $AlCl_3$ + P = 3.4, H-WTR = 9.2, $AlCl_3$ = 3.3, and H-WTR + P = 13.3.

[c] P intakes (g/(lamb d)): C = 2.0, L-WTR = 2.0, $AlCl_3$ + P = 3.8, H-WTR = 1.5, $AlCl_3$ = 1.5, and H-WTR + P = 3.8.

[d] Treatments are as follows: (1) control (n = 9), (2) low WTR (n = 8), (3) $AlCl_3$ + P (n = 7), (4) high WTR (n = 9), (5) $AlCl_3$ (n = 9), and (6) high WTR + P (n = 8).

[e] After week 4, lambs on H-WTR had lower (P < 0.05) intakes.

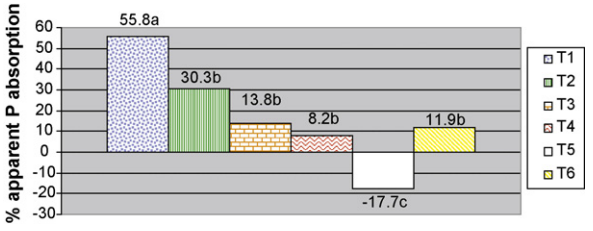

Fig. 1. Effects of dietary Al and P on apparent P absorption, started on d 34 with a 7 d collection. Dietary treatments were as follows: (1) control (n = 7), (2) low WTR (n = 7), (3) $AlCl_3$ + P (n = 7), (4) high WTR (n = 7), (5) $AlCl_3$ (n = 7), and (6) high WTR + P (n = 7). T2, T3, and T5 were formulated to contain 0.30% Al, T4 and T6 contained 1.2% Al. The S.E. for treatments is 8.72. [a,b,c]Means lacking a common superscript differ (P < 0.05); adjusted for multiple comparison.

consumed consistently less feed then other groups from week 6 through the end of the trial (Table 3).

Apparent absorption of Al varied from −21.7 to 8.6 in the first trial and −14.3 to 3.4 in the second trial (data not shown), suggesting that animals have a low ability to absorb Al. There were no treatment differences (P > 0.05) in apparent absorption of Al.

There were treatment differences (P < 0.05) in apparent absorption of P (Figs. 1 and 2). The first collection period began on d 34 (Fig. 1). All lambs receiving Al in their diet had decreased P absorption (L-WTR, 30.3%; $AlCl_3$ + P, 13.8%; H-WTR, 8.2%; $AlCl_3$, −17.7%; H-WTR + P, 11.9%) compared to control animals (C, 55.8%), and lambs fed $AlCl_3$ without added P supplementation had the lowest (P < 0.05) apparent P absorption, −17.7%. Although there were no statistical differences (P > 0.05) with time, lambs without added P had numerically lower apparent P absorption for the second collection. The second collection period began on d 70 (Fig. 2). Lambs receiving $AlCl_3$ + P and those receiving H-WTR had negative P absorption, −20.9 and −2.5%, respectively, and were not different (P > 0.05) from one another. Van Alstyne et al. (2007) found that a similar Al-based WTR did not decrease P absorption at 0.80% dietary Al when P was supplied at 0.25%, but apparent P absorption was −12.9% when Al was supplied as $AlCl_3$. The present data suggest that Al concentrations greater than or equal to 0.3%, regardless of source, greatly reduce the ability of lambs to absorb P. Others have reported the detrimental effects of Al as $AlCl_3$ on P absorption even when P is not limited in the diet (Valdivia et al., 1982; Van Alstyne et al., 2007).

Plasma concentrations of Al, Ca, Cu, Fe, Mg or Zn were not different (P > 0.05) at any collection date (data not shown). However, plasma P concentration was affected by treatment (Table 4). There were no differences in the d 0 samples, but at d 28 plasma P concentrations were lower (P < 0.05) for the H-WTR than for the control and added P diets (C, $AlCl_3$ + P, and H-WTR + P). Also, plasma concentrations for both the high and low WTR treatments (L-WTR and H-WTR) were below the critical level for plasma P, 45 μg/mL, by d 28 (McDowell and Arthington, 2005). By d 56, the only diet that was not different (P > 0.05) from C was $AlCl_3$ + P. All other treatments resulted in a decline in plasma P concentrations with L-WTR and H-WTR being the lowest; the treatments also resulted in the lowest plasma P concentration on d 84. Animals in the C treatment had higher (P < 0.05) plasma P concentrations then all other animals on d 98. Plasma P concentrations in the $AlCl_3$ + P group were not different (P > 0.05) from $AlCl_3$ and H-WTR + P; H-WTR + P in turn which were not statistically different from L-WTR. The H-WTR treatment again resulted in the lowest plasma P concentration. Aluminum appeared to affect the limited P in the diet, reducing P absorption. Animals on H-WTR also consumed only half of the daily feed provided which would affect total P intake. While the added P in H-WTR + P decreased the effects of the Al in WTR on

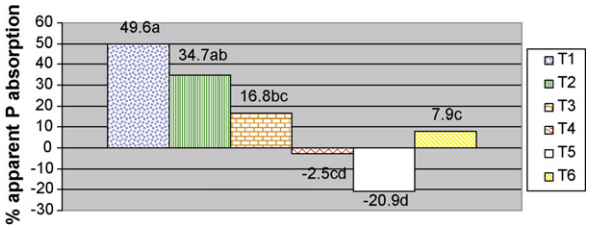

Fig. 2. Effects of dietary Al and P on apparent P absorption, started on d 70 with a 7 d collection. Dietary treatments were as follows: (1) control (n = 7), (2) low WTR (n = 7), (3) $AlCl_3$ + P (n = 7), (4) high WTR (n = 7), (5) $AlCl_3$ (n = 7), and (6) high WTR + P (n = 7). T2, T3, and T5 were formulated to contain 0.30% Al, T4 and T6 contained 1.2% Al. The S.E. for treatments is 8.72. [a,b,c,d]Means lacking a common superscript differ (P < 0.05); adjusted for multiple comparison.

Table 4
Effects of dietary Al and P on plasma P concentration (μg/mL)[a]

Day	Treatment[b]					
	1	2	3	4	5	6
0	52.4	49.8	54.6	51.2	56.1	56.2
28	65.4 a	33.8 bc	70.8 a	20.4 c	33.8 bc	43.0 b
56	72.2 a	39.3 bc	76.4 a	21.6 c	50.8 b	44.5 b
84	86.1 a	30.9 c	81.5 a	17.7 c	58.3 b	78.4 ab
98	87.8 a	39.4 c	67.9 ab	17.9 d	58.4 bc	53.8 bc
S.E.	5.43	5.75	6.15	5.43	5.43	5.75

[a] Means within rows lacking common letters (a,b) differ (P < 0.05); adjusted for multiple comparisons. The S.E. for day effect is 2.312.

[b] Treatments are as follows: (1) control (n = 9), (2) low WTR (n = 8), (3) $AlCl_3$ + P (n = 7), (4) high WTR (n = 9), (5) $AlCl_3$ (n = 9), and (6) high WTR + P (n = 8).

Table 5
Effects of dietary Al and P on soft tissue P concentration (% DM basis)[a]

Tissue[b]	Treatment[c]						S.E.
	1	2	3	4	5	6	
Heart	0.99	0.78	0.80	0.84	0.84	0.84	0.04
Kidney	0.95	0.90	0.89	0.82	0.98	0.83	0.03
Liver	0.74	0.90	0.92	0.78	0.86	0.81	0.02
Muscle	0.66	0.55	0.64	0.61	0.64	0.63	0.01

[a] No differences ($P > 0.05$) among treatments.
[b] Typical P concentrations in percentage (Miles et al., 2001) are as follows: heart, 1.0–1.1; kidney, 1.1–1.2; liver, 0.9–1.4; and muscle, 0.8–1.1.
[c] Treatments are as follows: (1) control ($n = 9$), (2) low WTR ($n = 8$), (3) $AlCl_3$ + P ($n = 7$), (4) high WTR ($n = 9$), (5) $AlCl_3$ ($n = 9$), and (6) high WTR + P ($n = 8$).

plasma P concentration when compared to those animals on H-WTR alone, it does not eliminate them when compare to no added dietary Al (C).

While plasma P concentrations were affected by concentrations of dietary Al and P, the tissue P concentrations were not. There were no differences ($P > 0.05$) in P concentrations in the heart, kidney, liver, or muscle (Table 5), and tissue P concentrations were relatively close to the normal ranges (Miles et al., 2001).

The tissue microelements concentrations exhibited few treatment differences (Table 6). The liver Cu concentrations were higher ($P < 0.05$) in $AlCl_3$ + P then in C and H-WTR + P animals, but not different ($P > 0.05$) from L-WTR, H-WTR, or $AlCl_3$ animals. The concentration of Al does not seem to be a factor in these Cu differences, as liver Cu concentrations in C and treatments with the highest concentration of Al (H-WTR and H-WTR + P) were not different ($P > 0.05$). Likewise, source of Al does not seem to be a factor as L-WTR and $AlCl_3$ with the same Al concentrations had similar liver Cu concentrations ($P > 0.05$). The cause for variation in liver Cu concentration in this study is unknown. Liver Fe concentrations were higher ($P < 0.05$) in the H-WTR treatment then in all other treatments. This is likely due to the high concentration of Fe in the WTR (Table 1). Liver Mn varied widely, with $AlCl_3$ + P resulting in concentrations lower ($P < 0.05$) than in L-WTR, $AlCl_3$, and H-WTR + P. Aluminum as $AlCl_3$, in combination with a low P diet, affects the concentration of Mn in the liver (Neathery et al., 1990).

Bone samples were collected at slaughter to determine the effect of dietary P and Al on bone minerals on a both a fresh and an ash basis. On a per unit volume basis (mg/cm^3, fresh basis), all mineral concentrations, except Al, were affected by treatment (Table 7). Sheep receiving the H-WTR treatment had lower ($P < 0.05$) bone Ca, P, and Mg concentrations than sheep from all other treatments, except for those receiving the lower WTR treatment (L-WTR). Lambs on L-WTR did not have different ($P > 0.05$) Ca, P, or Mg concentrations than the control or $AlCl_3$ lambs. Additional P supplementation appeared to counteract BMC loss caused by the high levels of Al supplied as WTR, as H-WTR + P had higher ($P < 0.05$) BMC then H-WTR. On an ash basis, only bone Ca concentration was affected by treatment. Sheep receiving H-WTR had the highest level of Ca. Magnesium, Al and P concentrations (ash basis) were not affected by treatment ($P > 0.05$) and were within the normal ranges (Miles et al., 2001; McDowell and Arthington, 2005). Literature suggests that bone mineral status of ruminants is more sensitive when expressed on a fresh basis (Little, 1972; Williams et al., 1990). In agreement, the present experiment showed differences in Ca, P, and Mg when measured on a fresh basis (mg/cm^3) but only for Ca when measured on an ash basis. Although not significant ($P > 0.05$) bone density (g/cm^3) and ash (%) were lowest for lambs receiving the H-WTR.

Radiographs taken on d 98 were compared to specific gravity (fresh basis) to determine if radiograph BMC (mm Al) could be correlated to the per unit volume (g/cm^3, fresh basis) and a significant ($P < 0.05$), but weak, correlation was found ($r = 0.59$). There were also significant ($P < 0.05$)

Table 6
Effects of dietary Al and P on soft tissue microelement concentration (mg/kg, DM basis)[a]

Mineral	Treatment[b]						S.E.
	1	2	3	4	5	6	
Al							
Brain	700	805	613	664	795	828	25.2
Heart	217	171	173	154	181	224	9.8
Kidney	130	106	92	141	121	114	5.5
Liver	543	608	535	596	686	662	17.6
Muscle	120	86	95	105	141	111	6.9
Cu							
Heart	21	19	19	18	21	20	0.68
Kidney	33	39	33	39	43	34	1.4
Liver	361 bc	436 abc	558 a	454 abc	514 ab	336 c	19.2
Muscle	10	10	12	11	10	12	0.03
Fe							
Heart	185	165	179	183	171	167	7.7
Kidney	153	183	385	257	200	171	33.7
Liver	165 b	173 b	164 b	357 a	164 b	172 b	11.5
Muscle	96	86	103	109	126	96	5.3
Mn							
Liver	10 bc	11 ab	8 c	10 abc	16 ab	12 a	0.3

[a] Means within rows lacking common letters (a,b) differ ($P < 0.05$); adjusted for multiple comparisons.
[b] Treatments are as follows: (1) control ($n = 9$), (2) low WTR ($n = 8$), (3) $AlCl_3$ + P ($n = 7$), (4) high WTR ($n = 9$), (5) $AlCl_3$ ($n = 9$), and (6) high WTR + P ($n = 8$).

Table 7
Effects of dietary Al and P on bone mineral concentration[a]

Item	Treatment[b]						S.E.
	1	2	3	4	5	6	
Density (g/cm^3)	1.87	1.85	1.88	1.81	1.88	1.85	0.01
Ca (mg/cm^3)	468 a	392 ab	463 a	316 b	429 a	446 a	12.1
P (mg/cm^3)	235 a	196 abc	228 ab	158 c	208 ab	209 ab	6.1
Mg (mg/cm^3)	7.6 a	6.1 ab	7.3 a	5.0 b	6.5 a	6.7 a	0.18
Al (μg/cm^3)	1.63	2.53	2.17	1.49	1.6	1.88	0.13
Ash (%)	69.1 ab	68.3 ab	69.4 a	68.0 b	69.3 ab	69.0 ab	0.14
Ca (%)	32.1 b	31.8 b	30.8 b	34.1 a	31.9 b	32.5 ab	0.23
P (%)	16.6	14.4	16.3	16.1	15.6	16.7	0.31
Mg (%)	0.53	0.49	0.48	0.53	0.48	0.49	0.01
Al (mg/kg)	1.2	2.1	1.4	1.6	1.2	1.5	0.11

[a] Means within rows lacking common letters (a,b) differ ($P < 0.05$); adjusted for multiple comparisons.
[b] Treatments are as follows: (1) control ($n = 9$), (2) low WTR ($n = 8$), (3) $AlCl_3$ + P ($n = 7$), (4) high WTR ($n = 8$), (5) $AlCl_3$ ($n = 9$), and (6) high WTR + P ($n = 8$).

Table 8
Effects of dietary Al and P on radiograph BMC over time (mm Al)[a]

	Treatment[b]					
	1	2	3	4	5	6
d 0	2.77	3.04	3.24	2.99	2.80	2.88
d 48	4.00	3.25	4.28	3.14	3.72	3.79
d 98	6.04[a]	4.78 ab	5.52 a	3.79 b	5.21 ab	5.11 ab

[a] Means within rows lacking common letters (a,b) differ ($P < 0.05$); adjusted for multiple comparisons. The S.E. for d 0 is 0.176 and for d 48 and 98 is 0.174.
[b] Treatments are as follows: (1) control ($n = 9$), (2) low WTR ($n = 8$), (3) $AlCl_3$ + P ($n = 7$), (4) high WTR ($n = 8$), (5) $AlCl_3$ ($n = 9$), and (6) high WTR + P ($n = 8$).

treatment differences in BMC (mm Al basis) for d 98 only (Table 8). The control diet (C) was the least affected and was different ($P < 0.05$) from the highest level of WTR only. Over time, the only animals that did not increase ($P > 0.05$) BMC from d 0 to d 98 were the animals on the H-WTR. While the high concentration of dietary Al likely caused the lower BMC, this is confounded by the fact that these animals were consuming just over half as much P as those on the other five treatments.

4. Conclusions

Animals receiving inadequate P can be detrimentally impacted by increased dietary Al concentrations, but P supplementation can counteract the negative Al effects. Lambs consuming 10% of their DM intake as WTR (total Al = 1.2%) and no supplemental P had lower performance and a decreased apparent P absorption. However, this level of dietary Al as WTR is greater than that which would be expected to occur when WTR is applied to land to decrease P runoff. A 2.5% by weight application of WTR approximates 25% surface coverage of a ha, and is sufficient to control P losses (O'Connor et al., 2002; Van Alstyne et al., 2007). Animals consuming 10% of their diets as soil amended with 25% WTR coverage per ha as their grazing diet would have a WTR intake of 2.5%. This approximates consumption per lamb of 0.31% Al as WTR if the WTR contains ~11.1% Al. In this study, lambs consuming the low WTR diet (0.30% Al) exhibited no detrimental effects from the Al, even though lambs received little P. Negative apparent P absorption was seen in lambs consuming the same concentration of Al as $AlCl_3$, which confirms the greater bioavailability of Al from soluble sources (e.g. $AlCl_3$) than from poorly soluble sources like WTR (Van Alstyne et al., 2007).

Two experiments by Madison (2007) applied the same WTR used in this experiment on pasture for grazing cattle. Over the course of 2 years, 75.8 metric tonnes/ha WTR was applied to the pastures with no effects on cattle performance. Results of the cattle experiments, and the present sheep experiment, demonstrate that WTR can be applied to pastures of grazing ruminants in sufficient quantities to decrease P runoff, with no detrimental effects to the animal.

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