Bone, blood and faecal response to an acidogenic lick for range cattle using different concentrations of ammonium chloride

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ABSTRACT

Thirty Brahman-Angus cross heifers at breeding age on extensive grazing were used to test the effectiveness of NH_4Cl as an acidogenic agent at 15, 18, 21 and 26 % of a dicalcium phosphate lick (A+), compared to a lick of 5 parts dicalcium phosphate and 3 parts salt (A–). Blood and faecal phosphorus (P), faecal calcium (Ca) and faecal magnesium (Mg) were significantly ($P < 0.05$) higher in A+ compared to A– animals at different stages of the experiment. Bone P did not increase in response to NH_4Cl but bone Ca was significantly ($P < 0.05$) higher in A+ animals offered 18 % NH_4Cl and significantly ($P < 0.05$) lower at 15 and 26 %. Bone Mg in A+ animals was significantly ($P < 0.05$) higher at 15 and 18 % NH_4Cl and lower at 26 % NH_4Cl compared to A– animals. Percentage ash in bone increased linearly from 62.5 to 64.9 % in A+ animals as NH_4Cl was increased in the lick from 15 to 21 % and then decreased to 63.3 % but was significantly ($P < 0.05$) greater than the percentage ash in A– animals when the NH_4Cl was increased to 26 %. Bone mineral status was only marginally improved by adding NH_4Cl to the lick in this study but blood P was significantly ($P < 0.05$) improved in A+ compared to A– animals when the lick contained 15, 21 and 26 % NH_4Cl. Based on bone mineral results reported here, it is recommended that, when NH_4Cl is used as an acidogenic agent in licks for range cattle, the amount in the lick should not exceed 21 %.

Key words: ammonium chloride, anions, bone mineral, calcium, Ca:P ratio, magnesium, phosphorus.

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INTRODUCTION

Despite the discovery of the connection between phosphorus (P) deficiency and botulism nearly 100 years ago[12], mineral deficiencies/imbalances related to P deficiency still exist today. Research that followed Theiler's discovery led to the recommendations for licks that are still used, but bone mineral imbalances continue to impact negatively on beef production despite the application of accepted lick formulations, perhaps because, according to de Waal *et al.*[6], the requirements of grazing beef cattle for supplementary P are still not known with certainty. In large areas of the Northern Cape and North West Provinces 10 % or more of the animals in a number of herds of range cattle suffer from a lameness that appears clinically to be due to osteomalacia, even with the provision of various commonly accepted lick formulations.

The effect of dietary anions on bone mineral, especially calcium (Ca), is well known. Goto[8] sacrificed calcium carbonate from bone in rabbits using 0.25N hydrochloric acid given by stomach tube. Oetzel *et al.*[10] showed that dietary anion-cation balance could affect systemic acid-base balance, and Dishington and Bjornstad[7] and Block[5] reported that a diet high in anions just prior to parturition could prevent milk fever either by improving Ca absorption from the gut or by increased blood Ca as a result of resorption of Ca from bone. Despite extensive research into the effects of acidogenic diets on Ca metabolism before the mid 1980s, there were no reports of research on the effects of acidogenic diets on P homeostasis.

Beighle *et al.*[1] first showed the positive effects of an anionic diet on P metabolism when calves on an acidogenic diet displayed increased serum inorganic P compared to calves on a cationic diet. A P-sparing effect of an acidogenic diet has been reported[3] in a study in which simultaneous increases in P concentrations in blood, bone and faeces were observed, demonstrating that an acidogenic diet can improve P utilisation in the bovine.

MATERIALS AND METHODS

On a farm in the North West Province on which about 10 % of the animals were showing lameness due to what appeared from gross pathology to be osteomalacia, 46 Brahman-Angus cross heifers were randomly assigned to 2 groups, A+ and A–, and grazed in adjoining camps with the same water supply. None of the animals in the 2 groups were showing any signs of lameness when the experiment began. From each of the 2 groups 15 heifers were chosen randomly, assigned numbers and used for sampling throughout the trial. Animals in the A– group were given a conventional lick consisting of 5 parts dicalcium phosphate and 3 parts salt. Animals in the A+ group were given a lick composed of dicalcium phosphate and NH_4Cl at 15, 18, 21 and 26 % concentrations at different stages of the experiment in an effort to find a concentration of NH_4Cl in the lick that would be acceptable to the animals and that would improve their P status as measured by blood, bone and faecal P. When the concentration of NH_4Cl in the A+ lick was changed, the animals were maintained on that lick for at least 5 weeks before blood, bone and faecal samples were collected. Table 1 gives the amount of lick consumed by the animals in both groups in grams per cow per day.

Cortical bone samples were collected under local anaesthesia from the right 10th, 11th and 12th and left 10th and 11th ribs at the middle location on the ribs using a 12.5 mm trephine. Collection, digestion and analysis of samples have been described previously[2,3].

Data were statistically analysed using the *t*-test for 2 samples assuming equal variances at a confidence level of $P < 0.05$.

RESULTS

When NH_4Cl was offered at 15 % of the dicalcium phosphate lick, animals on the anionic lick consumed nearly 3 times as

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Table 1: **Lick consumed by animals** (grams per cow per day).

NH_4Cl (%)	A+	A–
15	119	45
18	50	50
21	32	32
26	106	147

A+ = NH_4Cl plus dicalcium phosphate.
A– = 3 parts salt plus 5 parts dicalcium phosphate.

much lick as those on the conventional lick composed of salt and dicalcium phosphate (Table 1). When the lick contained 26 % NH_4Cl, both groups consumed an excessive amount of lick.

Preliminary investigations into the problem of lameness on the farm revealed that animals had abnormally high bone Ca (300–500 Mg/g fresh weight) and marginally low bone P (80–100 mg/g fresh weight) leading to a high bone Ca:P ratio (3–5) and softening of bone especially in the stifle joint.

Animals receiving NH_4Cl demonstrated a significant ($P < 0.05$) increase in blood P compared to A– animals when the lick contained 15, 21 and 26 % NH_4Cl (Table 2). In addition, A+ animals had significantly ($P < 0.05$) more faecal P compared to A– animals throughout the trial (Table 2). There were no significant ($P < 0.05$) differences in bone P between A+ and A– animals as NH_4Cl increased in the A+ lick from 15 to 21 %, but bone P decreased significantly ($P < 0.05$) compared to A– cows when NH_4Cl was offered at 26 % in the lick (Fig. 1).

Bone Ca was curvilinear in A+ animals, increasing when NH_4Cl was included at both 18 and 21 %, but decreasing in comparison to A– animals when the lick contained 26 % NH_4Cl (Fig. 2). The A+ animals had significantly ($P < 0.05$) more bone Ca at 18 % NH_4Cl but significantly ($P < 0.05$) less at 15 and 26 % NH_4Cl compared to A– animals (Table 3).

Faecal Ca had an inverse relationship to bone Ca increasing as bone Ca decreased and decreasing as bone Ca increased (Tables 3, 4), indicating that the Ca drawn from the bone was lost in the faeces.

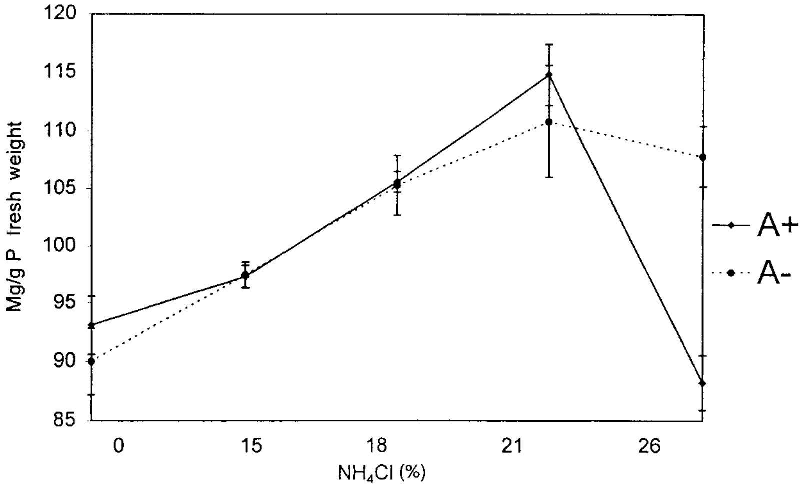

Fig. 1: **Bone phosphorus.**

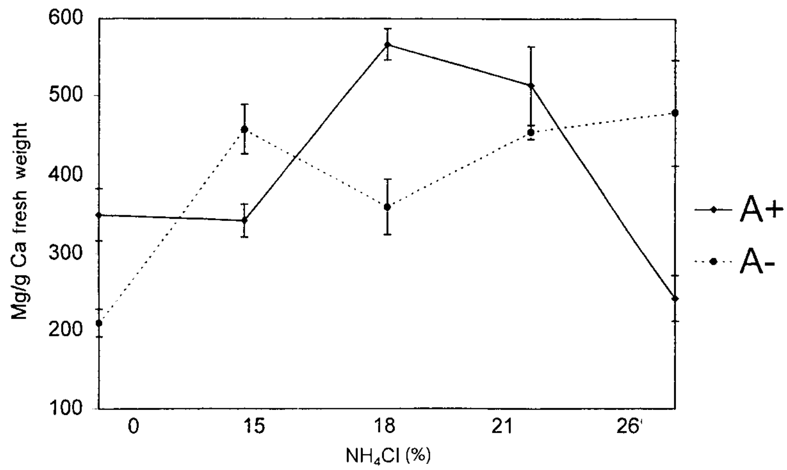

Fig. 2: **Bone calcium.**

The A+ animals had significantly ($P < 0.05$) more faecal Ca than A– animals at 15 % NH_4Cl but faecal Ca was not significantly ($P < 0.05$) different at other stages of the trial (Table 4). The greatest effect of the acidogenic lick on bone Ca resorption was seen at 15 and 26 % NH_4Cl with bone Ca absorption occurring at 18 % (Table 3).

The concentration of bone Mg was significantly ($P < 0.05$) higher in A+ animals compared to A– when the lick contained 15 and 18 % NH_4Cl but significantly ($P < 0.05$) lower at 26 % NH_4Cl (Table 3). Faecal Mg was significantly ($P < 0.05$) higher at 15 % and 21 % and significantly ($P < 0.05$) lower at 18 % NH_4Cl in A+ compared to A– animals (Table 4). As reported in the P results, a Mg-sparing effect was seen when both bone and faecal Mg increased as a result of the 15 % anionic lick.

Percentage ash in bone ranged from 62.5 % to 64.9 %, more than reported by Beighle *et al.*[8], but within the wide range reported by Read *et al.*[11]. The 63.3 % bone ash found in A+ animals receiving 26 % NH_4Cl was significantly ($P < 0.05$) more than the 62.6 % found in A+ animals during the same period. This was despite significantly ($P < 0.05$) lower bone Ca, P

Table 2: **Blood and faecal phosphorus.**

Lick	Blood				Faeces			
	A+		A–		A+		A–	
NH_4Cl (%)	Mg (%)	SEM	Mg (%)	SEM	Mg/g	SEM	Mg/g	SEM
0	2.8^a	0.22	2.3^a	0.17	3.0^a	0.15	3.9^b	0.31
15	11.0^a	0.25	8.7^b	0.25	3.7^a	0.35	1.4^b	0.04
18	9.4^a	0.38	9.1^a	0.41	1.7^a	0.11	1.0^b	0.05
21	11.9^a	0.81	5.7^b	0.25	1.6^a	0.07	1.3^b	0.05
26	11.4^a	0.31	9.2^b	0.15	4.4^a	0.31	2.7^b	0.11

[a,b]Means with the same letter are not significantly different ($P < 0.05$) between treatments.

Table 3: **Bone phosphorus, calcium and magnesium.**

Lick	Phosphorus Fresh weight				Calcium Fresh weight				Magnesium Fresh weight			
	A+		A–		A+		A–		A+		A–	
NH_4Cl (%)	Mg/g	SEM	Mg/g	SEM	Mg/g	SEM	Mg/g	SEM	Mg/g	SEM	Mg/g	SEM
0	93.1[a]	2.5	90.1[a]	2.8	350.1[a]	33.4	209.6[b]	18.1	18.9[a]	1.4	22.1[b]	1.8
15	97.3[a]	1.1	97.4[a]	1.1	354.1[a]	21.1	458.1[b]	30.9	18.4[a]	1.2	11.3[b]	0.9
18	105.0[a]	0.9	105.5[a]	2.6	566.2[a]	20.9	360.2[b]	35.1	18.4[a]	0.9	7.0[b]	0.8
21	114.1[a]	2.6	110.8[a]	4.8	513.5[a]	50.8	457.3[a]	9.1	12.6[a]	1.1	15.5[a]	0.8
26	88.2[a]	2.3	107.8[b]	2.6	242.1[a]	30.5	479.4[b]	67.1	13.6[a]	0.9	19.8[b]	2.4

[a,b]Means with the same letter are not significantly different ($P < 0.05$) between treatments.

Table 4: **Faecal calcium and magnesium.**

Lick	Calcium Fresh weight				Magnesium Fresh weight			
	A+		A–		A+		A–	
NH_4Cl (%)	Mg/g	SEM	Mg/g	SEM	Mg/g	SEM	Mg/g	SEM
0	6.9[a]	0.43	7.4[a]	0.41	1.9[a]	0.11	1.5[b]	0.09
15	13.5[a]	0.78	4.1[b]	0.32	2.2[a]	0.12	1.9[b]	0.13
18	5.6[a]	0.25	5.9[a]	0.51	1.0[a]	0.06	1.3[b]	0.06
21	5.4[a]	0.29	5.3[a]	0.45	1.0[a]	0.05	0.8[b]	0.07
26	11.4[a]	0.43	12.8[a]	0.84	2.2[a]	0.07	2.4[a]	0.13

[a,b]Means with the same letter are not significantly different ($P < 0.05$) between treatments.

and Mg concentrations in A+ compared to A– animals at the same period.

DISCUSSION

Higher intake of the anionic lick at 15 % NH_4Cl probably occurred because the concentration of NH_4Cl made the lick less salty than at higher concentrations of NH_4Cl. Excessive consumption occurred at 26 % NH_4Cl because the animals were on lush grazing compared to the other periods. The A+ animals consumed less lick than the A– animals because the 26 % lick was less palatable.

Previous research[3] has shown that a diet high in anions is capable of decreasing bone Ca while at the same time increasing bone P. This research was designed to determine if anions could be incorporated into a lick in sufficient quantities to bring about the same P-sparing effect that had been shown when anions were added to the feed[3]. The results agree with previous reports[3,4] of a P-sparing effect of an anionic diet with concurrent increased blood and faecal P in these animals on the acidogenic lick compared to animals on the control lick. It is unclear why bone P was not also increased in these animals, but it is noteworthy that when bone P was measured at about the same concentrations in both A+ and A– animals (Fig. 1), there were increases in both the blood and faecal P in A+ animals (Table 2), which could be explained only by the P-sparing effect of the acidogenic lick. Despite losses of P in the faeces, there

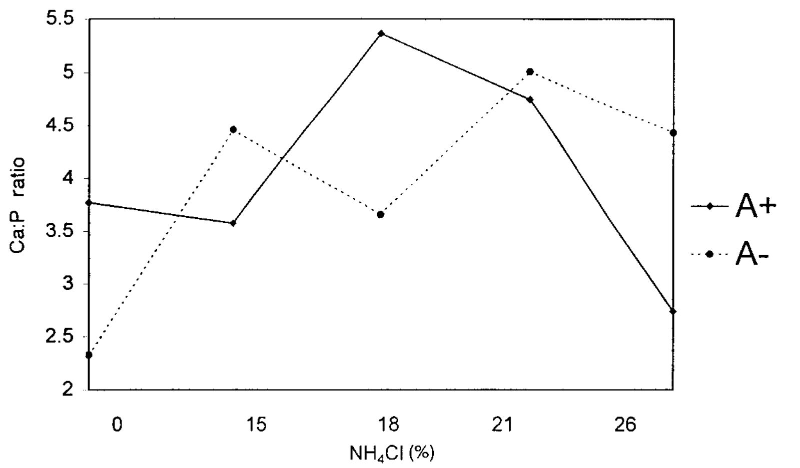

Fig. 3: **Ca:P ratio.**

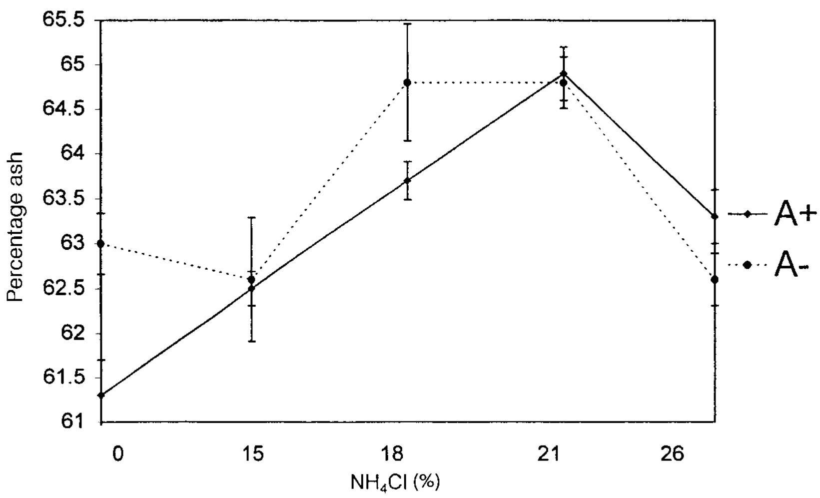

Fig. 4: **Percentage bone ash.**

were concurrent gains of blood P concentrations, significantly (P < 0.05) above those of animals not receiving the anions in the lick, as a result of the P-sparing effect.

Leclerc and Block[9] reported an increase in Ca being resorbed from bone as a result of an acidogenic diet. The present results agree, as an increase in the anions in the lick reduced the Ca in the bone at 2 different concentrations of NH_4Cl (15 %, 26 %) when compared to animals not receiving the anions. This decrease in bone Ca could have been beneficial in this study, decreasing the high Ca:P ratio, but at the same time the bone P was also decreased. This is contrary to results reported by Beighle *et al.*[3], who found an increase in bone P due to an acidogenic diet. The difference in the present study was the incorporation of the anions in the lick as opposed to a mixed ration in the earlier work. Herein lies the problem of increasing the anions in the diet of range cattle. Anions must be included in a lick and the amount that can be included in the lick is limited by the amount which is accepted by the animals. In addition, the anions are consumed along with high concentrations of Ca and P which no doubt impact on the effect of the anions. The dicalcium phosphate had more cations (Ca) than anions (P). Further research is needed to address this problem.

The goal of this experiment was to improve the Ca:P ratio by decreasing bone Ca using an anionic lick and at the same time increasing bone P due to the anionic lick. That occurred when the lick contained 26 % NH_4Cl, when both bone Ca and P were resorbed from bone (Table 3). Because bone Ca concentration decreased more than bone P, the Ca:P ratio was reduced to 2.74 for the A+ group compared to 4.43 for the A– group, closer to the desired 2 than at any other time in the experiment (Fig. 3). This decrease in the Ca:P ratio would have been more beneficial had the concentration of P in the bone not decreased. Although beneficial effects of NH_4Cl as an acidogenic agent in increasing blood P have been demonstrated in this study more work is needed to put together an anionic lick that will improve the bone P status of animals such as those in this experiment.

Results of this research agree with reports[4] that P resorption from bone is independent of Ca and Mg resorption. There were significant (P < 0.05) increases and decreases in bone Ca and Mg at certain stages of the experiment without corresponding changes in bone P (Table 3). Although these results are in agreement with previous studies using anions in mixed rations such as the P-sparing effect, there are some areas in which the animals did not respond to the acidogenic licks in the same way that they did to the acidogenic diets, such as by an increase in bone P. More research is needed to find the right combination of anions to bring about the increase in bone P as a result of the anionic diet reported earlier[3,4].

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