

## Bone mineral response to ammonium sulphate offered as a lick supplement in beef calves

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### ABSTRACT

Sixteen Bonsmara calves (4 males, 12 females) between 10 and 18 months of age were blocked according to age and sex and randomly assigned to 2 groups. They were offered licks containing bone meal and salt (50:50 ratio) (control) and bone meal and ammonium sulphate (NH<sub>4</sub>SO<sub>4</sub>) at 1.25, 2.5, 5, 10, 15, and 18 % (treatment) to evaluate the effects of dietary anions on bone phosphate (P) concentration. Bone P concentration was significantly ( $P < 0.05$ ) higher in the NH<sub>4</sub>SO<sub>4</sub> group compared with the control group, indicating that NH<sub>4</sub>SO<sub>4</sub> was able to increase the P content of bone at each of the 6 concentrations used in the lick relative to the control animals, thereby improving the P status of the animals. Ammonium sulphate at 15 % and 18 % in the lick also significantly ( $P < 0.05$ ) increased bone P compared with the lower concentrations of NH<sub>4</sub>SO<sub>4</sub>. Bone calcium (Ca) fluctuated as a result of the acidogenic lick. There was absorption of Ca when P was being resorbed and resorption of Ca when P was being absorbed into and out of bone. Bone Ca:P ratio ranged from 3.2 to 6.4 among the control group and 1.6 to 4.3 among the treatment group. Animals receiving the acidogenic lick had a higher percentage ash compared to the control group for most of the experimental period. Bone magnesium (Mg) fluctuated in response to the acidogenic lick, and it was difficult to show a relationship between bone Mg and Ca or P. The overall mean cortical bone thickness was significantly ( $P < 0.05$ ) greater in treatment (1.60 mm) compared with control (1.43 mm) calves and this was also true at sampling periods 2, 4, 5 and 6. Bone thickness followed bone P and not bone Ca. Results from this research indicate that the addition of ammonium sulphate to a lick had a beneficial effect in improving the P status by increasing bone P and improving the mineral status of bone by increasing the thickness of cortical bone and percentage ash.

**Key words:** ammonium sulphate, anions, bone, bone meal licks, calcium, magnesium, phosphorus.

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### INTRODUCTION

The role of the sulphate radical as an anion in the diet was recognised by Du Toit and Bisschop<sup>8</sup> when they reported that magnesium hydroxide bound calcium in the tissues while magnesium sulphate favoured its elimination. Eckles *et al.*<sup>9</sup> found that a high level of magnesium sulphate in the diet was responsible for a delay in the development of the typical signs of P deficiency, but when the magnesium sulphate was withdrawn from a diet low in P there was a rapid deterioration in the condition of the animals and a dramatic exhibition of pica.

Dishington<sup>7</sup>, while assigning 14 cows to a basic diet supplemented with Na<sub>2</sub>CO<sub>3</sub> during 4 weeks *pre-partum* and 1 week

*post-partum*, found that 12 of the 14 cows developed milk fever, while 12 cows of 13 receiving the same basic diet supplemented with sulphates and chlorides remained healthy. A mixture of CaCl<sub>2</sub>, Al<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub> and MgSO<sub>4</sub> were used to decrease the cation–anion balance to prevent milk fever. Jackson *et al.*<sup>12</sup> found that plasma Ca increased linearly with an increase in cation–anion balance.

In evaluating the acute effects of an acidogenic diet of –11.1 meq/100 g of diet dry matter, compared with a basogenic diet of +26.5 meq/100 g or a control diet of +16.5 meq/100 g of diet dry matter on blood, bone and faecal P, Ca and Mg for a period of 9 weeks, Beighle *et al.*<sup>3</sup> demonstrated a resorption of Ca from bone with increased blood Ca in response to an acidogenic diet, but a simultaneous increase in bone, blood and faecal P concentrations at various stages of the experiment as a result of the acidogenic diet. Vitti

*et al.*<sup>15</sup> reported that bone resorption of P was not influenced by the intake of P but did play an important role in the homeostatic control of P.

The research reported on in this paper was carried out to evaluate the effects of different concentrations of NH<sub>4</sub>SO<sub>4</sub> as an acidogenic agent in improving the effectiveness of bone meal licks in increasing bone P and improving the P status of the bovine, in comparison with conventional bone meal plus salt licks.

### MATERIALS AND METHODS

Sixteen Bonsmara calves (4 males, 12 females) between 10 and 18 months of age were blocked according to age and sex and randomly assigned to 2 groups of 8 animals each. Calves were used instead of adults in an effort to monitor short-term changes in bone mineral. The animals were confined in experimental enclosures 15 × 15 m with concrete floors and shade being provided. Water was freely available at all times at automatic water troughs. Roughage was given *ad libitum* and was composed of 50 % blue buffalo grass (*Cenchrus ciliaris*) and 50 % lucerne. On a dry matter basis the roughage contained 2.6 mg P/g, 3.3 mg Ca/g and 0.6 mg Mg/g. A separate trough was provided for the licks, which were fed once daily with weigh backs recorded at each sampling day. The amount of lick consumed is given in Table 1. Acidogenic licks were formulated by the addition of NH<sub>4</sub>SO<sub>4</sub> to bone meal at 1.25, 2.5, 5, 10, 15 and 18 % of the lick. The concentration was increased each 10 days. The control lick was formulated by the addition of sodium chloride to bone meal at a 50:50 ratio (Table 1.). At sampling period 3 when the animals were offered NH<sub>4</sub>SO<sub>4</sub> at 5 % of the bone meal lick the animals refused to consume the lick, most likely because of the increasing concentration of the ammonium sulphate. It was therefore necessary to add dried molasses to the subsequent licks of both the control and treatment groups at a rate of 5 % to encourage the animals to consume the lick so that both groups of calves received about the same amount of bone meal. The dried molasses would have added

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Table 1. Lick consumed.

Sampling period:	SP 0	SP 1	SP 2	SP 3	SP 4	SP 5	SP 6
% NH <sub>4</sub> SO <sub>4</sub> in lick	0	1.25	2.5	5	10	15	18
Lick consumed <sup>1</sup>	kg/day	kg/day	kg/day	kg/day	kg/day	kg/day	kg/day
Treatment	0	1.5	0.75	0	0.25	0.5	0.25
Control	0	0.325	0.325	0	0.25	0.320	0.25
NH <sub>4</sub> SO <sub>4</sub> consumed <sup>2</sup>	g/day	g/day	g/day	g/day	g/day	g/day	g/day
Treatment group	0	2.3	2.3	0	3.1	9.4	5.6

<sup>1</sup>Kilograms consumed by 8 calves (each group) each day in the treatment and the control group.

<sup>2</sup>Grams of ammonium sulphate consumed by each animal each day in the treatment group.

potassium to the lick and this would have increased the basogenic nature of the lick. This explains why the greatest response to the acidogenic lick was at the higher concentrations of ammonium sulphate when the basogenic effect of the potassium would have been overcome by the acidogenic effect of the ammonium sulphate. At the same time the control animals also refused the lick because of rain damage. As can be seen in Table 1 the animals on the acidogenic lick consumed about the same amount of lick at sampling periods 4, 5 and 6 as those on the control lick. Ammonium sulphate was added at different percentages to the lick of the treatment group of 8 animals at 10 day intervals in an effort to find the concentration which would have the greater effect on bone mineral concentration. Bone is a much more dynamic tissue than previously thought. Weekly changes in bone Ca and P concentrations have been reported<sup>3</sup>. In addition, long-term changes in bone mineral have been reported<sup>5</sup> with different concentrations of ammonium chloride. This research was designed to evaluate the short-term effects of different concentrations of NH<sub>4</sub>SO<sub>4</sub> on bone mineral, especially P.

Rib bone samples were taken at the beginning of the trial before the animals were given the lick and throughout the experiment at 10 day intervals from the middle of the ribs, beginning with the 9th rib on the left side and continuing with the 10th, 11th and 12th ribs. Sampling then continued with the right 9th, 10th and 11th ribs of the calves.

Rib bone samples were surgically collected by using a trephine to remove a 12.5 mm circular core. In preparation for surgery the hair was shaved over the rib to be sampled, the area was surgically prepped, 10 ml of 2 % lignocaine was injected and a 3 cm incision was made in the skin over the rib and carried down through the muscle and the periosteum to the bone. With proper movement of the trephine a sample of bone was collected from the rib. Sandpaper was used to completely remove the trabecular bone that might have been taken out with the bone

sample, leaving only the cortical bone for thickness measurement and for use in P, Ca and Mg analysis. A calliper was used to measure the cortical bone thickness in millimetres. Trabecular bone was removed because it contained a variable number of red blood cells that contained P. Because the amount of trabecular bone was inconsistent in a biopsy sample, the number of erythrocytes varied in each sample<sup>3</sup>. Trabecular bone was removed to eliminate inconsistency in the P values due to the P in the erythrocytes of the trabecular bone. Cortical bone samples were processed as previously described<sup>1</sup>.

Bone samples were analysed colorimetrically at 420 nm for P, 570 nm for Ca and 630 nm for Mg by the method of Fiske and Subarrow<sup>10</sup> and Kaplan and Szabo<sup>13</sup> using a Bran & Luebbe Auto-Analyzer II: Technicon Industrial Systems, Tarrytown New York.

Data were analysed using Minitab 13.13 (2000). One-way analysis of variance (ANOVA) was done to determine whether the application of ammonium sulphate as an acidogenic agent in the lick can have a significant effect on the total P, Ca and Mg of the bone. Co-variance analysis was used where there was a wide difference between the treatment and the control groups especially during sampling period zero. The significant differences between treatments means were determined by Duncan's new multiple range test<sup>14</sup>.

## RESULTS AND DISCUSSION

There are indications that the acidogenic lick was responsible for significantly ( $P < 0.05$ ) higher concentrations of cortical bone P at all sampling periods (sp) compared with the animals receiving the control lick (Table 2). The P content of bone from the treatment group significantly ( $P < 0.05$ ) increased from 70.53 mg P/g (sp0) to 131.05 mg P/g (sp1) and then significantly ( $P < 0.05$ ) increased again to 157.07 mg P/g (sp 6), on a dry weight basis. In addition, when animals were receiving the lick containing 15 % and 18 % NH<sub>4</sub>SO<sub>4</sub>, they had significantly ( $P < 0.05$ ) higher concentrations of cortical bone P than when they were given lower concentra-

tions of NH<sub>4</sub>SO<sub>4</sub>. The highest concentration of P in the bone on a dry weight basis was 157.07 mg P/g when 18 % NH<sub>4</sub>SO<sub>4</sub> was given in the lick and the lowest was 94.53 mg P/g when 5 % NH<sub>4</sub>SO<sub>4</sub> was offered in the lick of the calves on the acidogenic lick and they refused to consume the lick, but in the calves on the control lick the highest mean was 124.27 mg P/g and the lowest mean was 68.45 mg P/g (Table 2). Some of the means were slightly higher than those from Beighle *et al.*<sup>1,2</sup>, who reported an overall mean of 107.88 and 108.25 mg P/g dry weight in rib bone of cattle.

The mean cortical bone thickness of animals on the acidogenic lick was significantly ( $P < 0.05$ ) higher at all sampling periods with the exception of sampling periods 1 and 3 compared with the animals on the control lick. At sampling period 1 the percentage NH<sub>4</sub>SO<sub>4</sub> was very low and at sampling period 3 the animals refused the lick. The highest mean among the animals given the acidogenic lick was seen at week 6 when 18 % NH<sub>4</sub>SO<sub>4</sub> was given in the lick (2.14 mm) as compared with 1.45 mm from animals on the control diet (Table 2). These values are only slightly lower than previous reports<sup>4</sup> of 1.90 mm to 3.43 mm in adult dairy cattle where higher values would be expected because of their age<sup>2</sup>.

Cortical bone thickness responded faster to the effects of the ammonium sulphate and the lick than bone P. At sampling period 3 when animals failed to consume the acidogenic lick, bone thickness decreased and was significantly ( $P < 0.05$ ) less than bone thickness from control animals. Bone P, however, at the same sampling period, was still significantly ( $P < 0.05$ ) higher compared with control animals. At sampling period 4 when the animals were again consuming the acidogenic lick, their mean bone thickness increased and was significantly ( $P < 0.05$ ) greater than the mean bone thickness from the control animals (Table 2). Bone P from the treatment group, however, remained significantly ( $P < 0.05$ ) above the control values at sampling periods 2, 3 and 4 (Table 2).

Table 2: The effect of ammonium sulphate on bone P, Ca and Mg concentration, % ash, Ca:P ratio and bone thickness.

Sampling period: Lick concentration:	SP 0 0		SP 1 1.25 % AS		SP 2 2.5 % AS		SP 3 5 % AS		SP 4 10 % AS		SP 5 15 % AS		SP 6 18 % AS	
<b>% Ash</b>	<b>%</b>	<b>SEM</b>	<b>%</b>	<b>SEM</b>	<b>%</b>	<b>SEM</b>	<b>%</b>	<b>SEM</b>	<b>%</b>	<b>SEM</b>	<b>%</b>	<b>SEM</b>	<b>%</b>	<b>SEM</b>
Treatment	62.4 <sup>a</sup>	0.003	69.1 <sup>a</sup>	0.008	69.4 <sup>a</sup>	0.972	61.7 <sup>a</sup>	0.005	60.8 <sup>a</sup>	0.004	62.4 <sup>a</sup>	0.004	62.4 <sup>a</sup>	0.003
Control	61.7 <sup>b</sup>	0.005	69.7 <sup>b</sup>	0.013	66.1 <sup>b</sup>	0.019	62.3 <sup>b</sup>	0.004	64.2 <sup>b</sup>	0.004	61.7 <sup>b</sup>	0.005	61.7 <sup>b</sup>	0.005
<b>CBNP (dry weight)</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>
Treatment	70.53 <sup>wx</sup>	23.2	131.05 <sup>xy</sup>	46.3	114.81 <sup>xy</sup>	22.4	94.53 <sup>xy</sup>	28.8	103.67 <sup>xy</sup>	36.4	131.94 <sup>yz</sup>	28.9	157.07 <sup>yz</sup>	50.9
Control	115.09 <sup>a</sup>	38.0	107.96 <sup>b</sup>	32.7	90.77 <sup>b</sup>	21.2	79.85 <sup>b</sup>	34.4	68.45 <sup>b</sup>	18.4	105.44 <sup>b</sup>	15.7	124.27 <sup>b</sup>	16.8
<b>CBNCa (dry weight)</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>
Treatment	196.14 <sup>a</sup>	0.22	293.92 <sup>a</sup>	0.32	243.39 <sup>a</sup>	0.15	317.45 <sup>a</sup>	0.32	442.52 <sup>a</sup>	0.42	310.41 <sup>a</sup>	0.44	249.00 <sup>a</sup>	0.30
Control	152.83 <sup>a</sup>	0.01	344.80 <sup>b</sup>	0.75	350.54 <sup>b</sup>	0.87	353.01 <sup>b</sup>	0.85	411.38 <sup>a</sup>	0.88	485.92 <sup>b</sup>	0.90	411.04 <sup>b</sup>	0.88
<b>CBNMg (dry weight)</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>	<b>mg/g</b>	<b>SEM</b>
Treatment	9.13 <sup>a</sup>	0.92	18.27 <sup>a</sup>	1.83	12.48 <sup>a</sup>	1.12	14.65 <sup>a</sup>	1.34	13.75 <sup>a</sup>	1.25	16.25 <sup>a</sup>	1.75	20.56 <sup>a</sup>	2.56
Control	9.22 <sup>a</sup>	0.98	15.22 <sup>b</sup>	1.68	13.44 <sup>a</sup>	1.23	14.05 <sup>a</sup>	1.31	16.07 <sup>b</sup>	1.77	13.28 <sup>b</sup>	1.27	23.90 <sup>b</sup>	2.99
<b>Ca:P ratio</b>														
Treatment	2.7		2.2		2.1		3.3		4.3		2.4		1.6	
Control	1.3		3.2		3.9		4.4		6.4		4.6		3.3	
<b>CBNTH</b>	<b>mm</b>	<b>SEM</b>	<b>mm</b>	<b>SEM</b>	<b>mm</b>	<b>SEM</b>	<b>mm</b>	<b>SEM</b>	<b>mm</b>	<b>SEM</b>	<b>mm</b>	<b>SEM</b>	<b>mm</b>	<b>SEM</b>
Treatment	1.57 <sup>a</sup>	0.18	1.36 <sup>a</sup>	0.16	1.58 <sup>a</sup>	0.08	1.46 <sup>a</sup>	0.17	1.56 <sup>a</sup>	0.17	1.51 <sup>a</sup>	0.18	2.14 <sup>a</sup>	0.15
Control	1.45 <sup>b</sup>	0.17	1.54 <sup>b</sup>	0.13	1.44 <sup>b</sup>	0.19	1.57 <sup>b</sup>	0.18	1.37 <sup>b</sup>	0.18	1.21 <sup>b</sup>	0.14	1.45 <sup>b</sup>	0.23

<sup>a,b</sup>Means with different letters in a column are significantly different between treatment groups ( $P < 0.05$ ).

<sup>w,x,y,z</sup>Means with different letters in a row are significantly different within treatment groups ( $P < 0.05$ ).

AS = ammonium sulphate, CBNTH = Cortical bone thickness, CBNMg = cortical bone magnesium, CBNCa = cortical bone calcium, CBNP = cortical bone phosphorus, mm = millimetre, SEM = standard error of mean.

It was expected that both mean cortical bone thickness and mean cortical bone P would be less in treatment animals compared with control animals when the treatment animals failed to consume the lick at sampling period 3. Bone thickness was less but bone P was greater in treatment compared with control animals. This indicates that the bone thickness changed faster than the bone P concentration. During the 10 days when the animals were not ingesting any lick, their mean bone thickness decreased so much that it was significantly ( $P < 0.05$ ) less compared with the control animals. During the same 10 days the mean bone P remained significantly ( $P < 0.05$ ) greater compared to the control animals. Further evidence for the rapid change in bone thickness is the increase in the mean bone thickness at sampling period 4 so that it was again significantly ( $P < 0.05$ ) greater compared with the control animals (Table 2).

Calves in the treatment group were not receiving any  $\text{NH}_4\text{SO}_4$  or lick P during sampling period 3 but bone P remained above that of the control group, probably from the effect of the previous treatment but, compared with the control group, bone thickness was reduced (Table 2). This would indicate that the bone thickness parameter responded to the absence of lick  $\text{NH}_4\text{SO}_4$  and P faster than the bone P parameter.

Bone Ca was significantly ( $P < 0.05$ ) lower in the cortical bone of the calves in

the treatment group compared to the control group at all sampling periods except sampling period 4 (Table 2). This is consistent with Block<sup>6</sup> who found increasing blood Ca in response to an acidogenic diet, which could have come from the bone. Bone Ca concentrations exceeded pre-treatment values at all sampling periods for both groups and ranged from 243.39 mg Ca/g to 485.92 mg Ca/g on a dry weight basis. This was probably because the animals had not been given bone meal prior to the experiment and they were using the Ca to replenish bone stores.

The concentration of P and Ca in the cortical bone responded to the acidogenic

lick differently at different concentrations of ammonium sulphate. The trend was for an increase in cortical bone Ca when cortical bone P decreased and a decrease in cortical bone Ca when cortical bone P increased (Fig. 1). Between sampling periods 2 and 3 the P concentration in the cortical bone decreased but the Ca concentration increased and between sampling periods 4 and 5 and 5 and 6 the P concentration increased but the Ca concentration decreased compared with the previous sampling period (Fig. 1). This confirms previous research in which an independent movement of Ca and P into and out of cortical bone has been

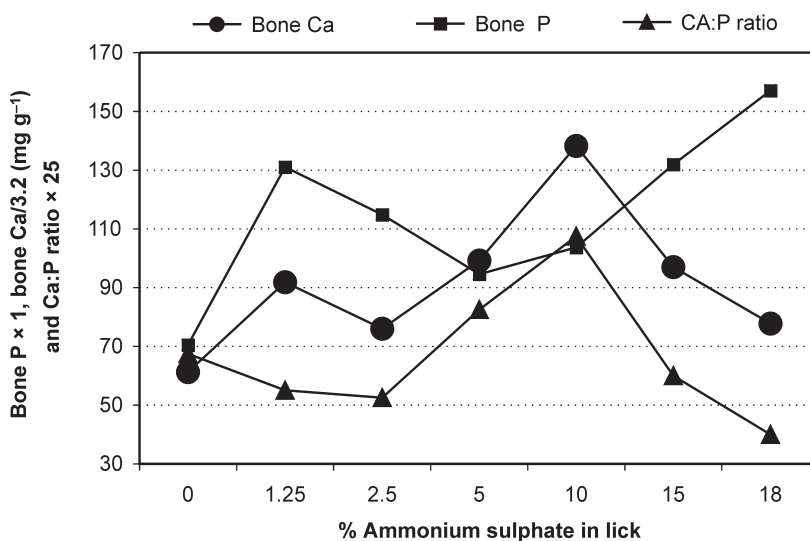


Fig. 1: Relationship between bone P, Ca and Ca:P ratio in animals receiving an acidogenic lick.

reported<sup>3,11</sup>.

The bone Ca:P ratio ranged from 1.6 to 4.3 among animals receiving the acidogenic lick but varied widely between 1.3 and 6.4 among animals receiving the conventional lick (Table 2). Only when the animals were receiving the ammonium sulphate at 5 and 10 % in the lick did the Ca:P ratio greatly exceed the normally accepted value of 2, whereas among the animals receiving the bone meal and salt lick the Ca:P ratio exceeded 2 throughout the experiment except for the pre treatment samples at sampling period 0. Throughout the trial the animals on the acidogenic lick were able to maintain a Ca:P ratio below that of the control group (Fig. 3) in agreement with Beighle *et al.*<sup>3</sup> who found that an acidogenic lick can assist animals in maintaining a more constant Ca:P ratio, by increasing cortical bone P and decreasing cortical bone Ca.

The cortical bone Ca:P ratio followed bone Ca and not P (Fig. 1) in agreement with Beighle *et al.*<sup>3</sup>. This has implications in formulating licks, which address the problem of P deficiency. The tendency of P to respond to an acidogenic lick in a different way compared to Ca allows us to use anions in a lick to improve the P status of the animal at the same time that Ca is resorbed from the bone. In situations where a high bone Ca:P ratio threatens the normal P homeostatic mechanisms an acidogenic lick can improve the bone Ca:P ratio by increasing bone P when bone Ca is decreasing.

Among animals receiving the acidogenic lick the bone Ca:P ratio varied more widely than the cortical bone thickness (Fig. 2). While the Ca:P ratio tended to follow Ca the cortical bone thickness tended to follow P especially at the higher concentrations of ammonium sulphate (Fig. 1, 2). Cortical bone thickness is not often used as a parameter in evaluating mineral status but results reported here confirm previous reports<sup>4</sup> and warrant further investigation into the use of this important parameter in the evaluation of the mineral status in cattle.

The response of bone Mg to the acidogenic lick was inconsistent. When the lick contained 1.25 % and 15 % ammonium sulphate, animals given the acidogenic lick had significantly ( $P < 0.05$ ) more cortical bone Mg than those animals on the control lick, but at sampling periods 4 and 6 when the acidogenic lick contained 10 and 18 % ammonium sulphate, the animals offered the control lick had significantly ( $P < 0.05$ ) more cortical bone Mg than those on the acidogenic lick (Table 2). This is in agreement with Beighle<sup>5</sup> who also found that bone Mg was inconsistent in responding to an

Table 3. Ingredients and nutrient composition of experimental diets.

Ingredients	Bone meal* (%)	Molasses* (%)
Crude protein	24.5	10.7
P	11.9	0.29
Ca	26.8	0.87
Na	0.79	0.19
K	0.19	3.68
Cl	0.10	—
S	0.13	0.46
Moisture	6.0	4.0

\*Dry matter basis.

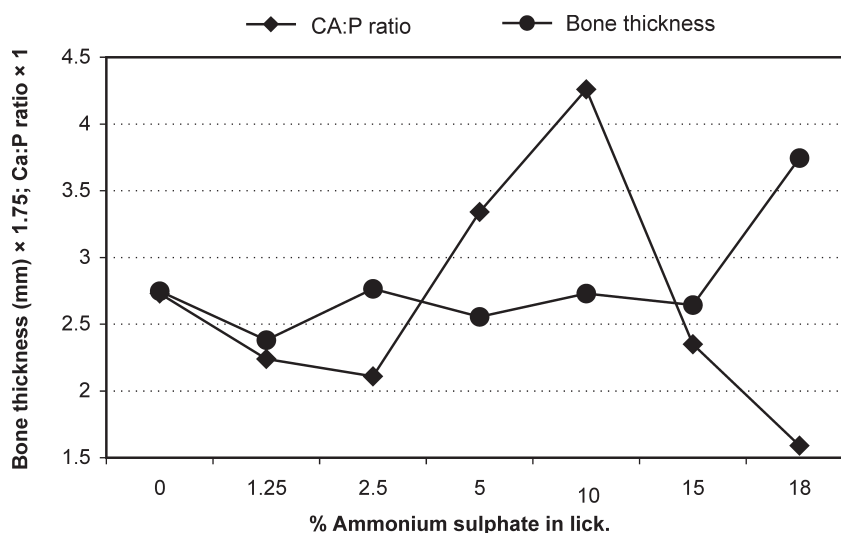


Fig. 2: Relationship between bone thickness and Ca:P ratio in animals receiving an acidogenic lick.

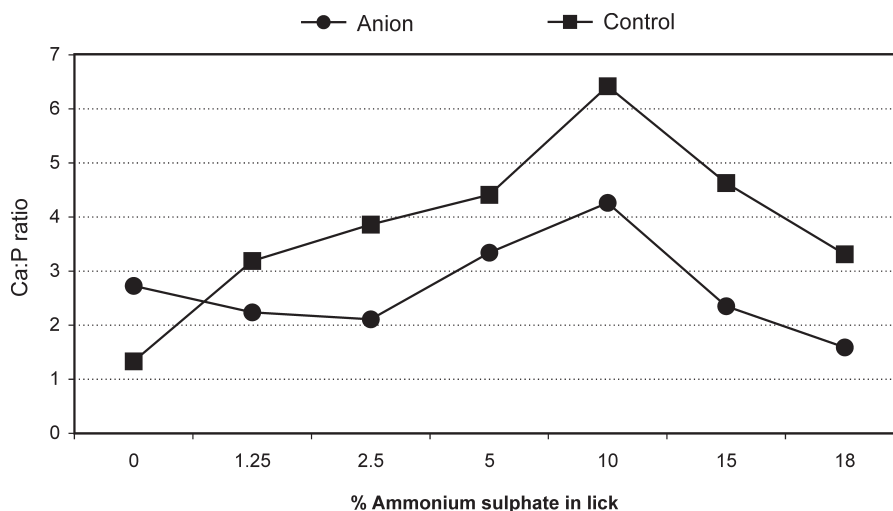


Fig. 3: Ca:P ratio in animals receiving an acidogenic lick compared with those receiving a bonemeal lick.

acidogenic lick composed of ammonium chloride, with increasing bone Mg at 15 and 18 % ammonium chloride but decreasing bone Mg at 26 % ammonium chloride.

Bone Mg was also unpredictable when compared to the other 2 mineral parameters. Bone Mg fluctuated in response to the anionic lick based on significant ( $P < 0.05$ ) differences at sampling periods 1, 4, 5 and 6. It was therefore difficult to build a relationship between bone Mg and Ca or

P. At the lower concentrations of ammonium sulphate the concentrations of Mg and Ca in bone responded in the same direction, but at the higher concentrations of ammonium sulphate their response was in opposite directions. At sampling periods 1, 2, 5 and 6 the concentrations of Mg and P responded to the acidogenic lick in the same way, but at weeks 3 and 4 they responded in opposite directions. Bone Mg increased in compari-

son to the previous sampling and bone P decreased at sampling period 3. Bone Mg decreased at sampling period 4 compared to sampling period 3 but bone P increased at sampling period 4 compared to sampling period 3 (Table 2).

The percentage ash of cortical bone was significantly ( $P < 0.05$ ) higher in the animals receiving the ammonium sulphate compared with that from the control group at sampling periods 2, 5 and 6 (Table 2). This might indicate that the failure of the treatment group to consume any lick at sampling period 3 had an impact on the bone ash even in sampling period 4. The treated animals consumed more lick than control animals at sampling period 2 and this could have caused the difference in percentage ash, but the treatment animals also consumed more lick in sampling period 1 and the percentage ash was significantly ( $P < 0.05$ ) higher in the control animals. These results indicate that the most consistent improvement of bone percentage ash was when the concentration of ammonium sulphate was at 15 % and 18 % based on significant ( $P < 0.05$ ) differences at sampling periods 5 and 6.

Results reported here confirm the dynamic nature of bone and the ability of bone to store P in the face of Ca resorption<sup>3,5,11</sup>. This research suggests that the inclusion of ammonium sulphate in a conventional bone meal lick improves the P status of the animal as reflected by the increase in cortical bone P at all concentrations of ammonium sulphate in the lick but especially at 15 and 18 %. Vitti *et al.*<sup>15</sup> reported that bone resorption of P plays an important role in the homeostatic control of P. This research shows that

bone P absorption occurs in response to an acidogenic lick and could play an even more important role in the P homeostatic mechanisms. In addition, this research shows that acidogenic licks can increase the ash content of cortical bone, the thickness of the cortical bone and decrease the Ca:P ratio in cortical bone.

The results of this research indicate that the best lick combination for increasing cortical bone P is a 15 % or 18 % ammonium sulphate + bone meal lick. Further research is needed to investigate the formulation of an acidogenic lick that can promote an increasing bone P with a concurrent increase in blood P and a decrease in faecal P.

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