Acute effects of an anionic diet on bone mineral homeostasis in the bovine

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ABSTRACT

Fifteen Friesian oxen between 12 and 18 months of age with a mean body mass of 240.7 kg were randomly assigned to diets containing 0.25 % phosphorus (P) or less, to evaluate the acute effects of an acidiogenic diet of –11.1 meq/100 g of diet dry matter, compared with a basiogenic diet of +25.6 meq/100 g or a control diet of +16.5 meq/100 g of diet dry matter calculated as (Na + K) – (Cl + S), on blood, bone and faecal P, calcium (Ca) and magnesium (Mg) for a period of 9 weeks. Blood, bone and faecal responses to an anionic diet are described. An inverse relationship existed between bone and blood Ca, in which there was resorption from bone with increased blood Ca in response to the anionic diet. The anionic treatment group demonstrated simultaneous increases in bone, blood and faecal P concentrations at various stages of the experiment compared to the cationic and control treatment groups. Results indicate independent absorption and resorption of Ca and P into and out of bone. There was wide variation in the bone Ca:P ratio between 2.02 and 1.51 among animals fed the anionic diet, with the Ca:P ratio following Ca values and not bone P values. Bone and blood P had a linear relationship with dietary cation:anion balance (DCAB), increasing as the diet became more anionic in nature, but faecal P was curvilinear with highest concentrations at –11.1 and +25.6 meq/100 g compared to +16.5 meq/100 g. Concurrent blood, bone and faecal P increases at some stages of the experiment indicate a P-sparing effect of the anionic diet and warrants further research into the long-term effects of anions in the diet, leading to their use as a possible addition to improved licks in P-deficient areas.

Key words: anionic, Ca:P ratio, calcium, cationic, magnesium, phosphorus.

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INTRODUCTION

Large areas of South African pasture produce phosphorus (P)-deficient herbage as a result of P-deficient soils[11,14]. As a result, cattle are forced to subsist on P-deficient diets for large parts of the year, especially in drought periods. Current licks used to supplement this P deficiency make use of either bonemeal, monocalcium phosphate, or dicalcium phosphate, in combination with various other ingredients including salt.

The beneficial effects of a diet high in anions on growth rate in dairy calves as a result of improved P utilisation when P was limiting[1], prevention of milk-fever[6,10] and storage of bone P[2] have been reported. More recently it has been shown that weekly monitoring of bone mineral values allows for more accurate assessment of bone Ca and P absorption and resorption in the bovine[4], based on research that showed that serial samples from ribs 9, 10, 11 and 12 from the right and left sides in the bovine may be compared[3]. Sampling on a weekly basis can prevent erroneous results. It has been shown that less frequent sampling can lead to conflicting conclusions of either a decline or an increase in bone P depending on when the samples are taken, especially when very acute changes are expected[4]. The purpose of this article is to report further on the effects of an anionic diet on mineral homeostasis, especially Ca and P, by monitoring mineral values on a weekly basis.

MATERIALS AND METHODS

Materials and methods used in this research have been described previously[4]. Data were analysed using the Statistical Analysis System[12]. A randomised block design analysis of variance was used. A regression analysis was used to compare relationships between blood, bone and faeces and to compare effects of dietary cation:anion balance (DCAB) on blood, bone and faecal P, where linear and non-linear regression analysis was used. For weekly comparisons, the repeated measures analysis of variance was used that also dealt with comparisons among treatments.

RESULTS

Weekly mean P concentrations found in blood, bone and faeces from animals in the anionic treatment group are shown in Fig. 1. When compared with pre-treatment values (week 0), blood, bone and faecal concentrations of P increased simultaneously during the 1st 2 weeks among the anionic treatment group. Blood P remained above pre-treatment values throughout the trial, significant ($p < 0.02$) at weeks 2, 3, and 8. Faecal P remained above pre-treatment values throughout the trial but was not significant between weeks. Bone P values were above those of pre-treatment at weeks 1, 2, 3, 6, and 8 significantly ($p < 0.02$) above week 0 or the previous week at weeks 2 and 8 (Fig. 1).

Animals in the anionic treatment group had significantly ($p < 0.02$) more bone P than those in the cationic treatment group at weeks 2 (126 *vs* 120 mg/g) and 9 (108 *vs* 100 mg/g) on a dry weight basis, and tended to have higher bone P throughout the trial. With the exception of weeks 4 and 7, the animals in the anionic treatment group tended to have higher bone P than those in the control treatment group.

Animals in the anionic treatment group had significantly ($p < 0.02$) more faecal P than animals offered the control diet at weeks 4 (38.5 *vs* 28.6 mg/g), 6 (38.1 *vs* 31.1 mg/g) and 8 (37.0 *vs* 23.3 mg/g) measured on an ash weight basis. The same animals tended to have higher faecal P from weeks 2–9.

Blood P from the anionic treatment group was significantly ($p < 0.05$) greater at week 2 (10.4 *vs* 7.8 mg%) than that from the control treatment group, and tended to be higher in the anionic compared to the control treatment group at weeks 1, 2,

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Table 1: **Mean calcium concentrations in faeces measured as mg/g dry weight.**

	Treatment group					
	Anionic		Cation		Control	
Week		SEM		SEM		SEM
0	$18.78^{a\,z}$	3.90	$11.91^{a\,yz}$	2.06	$16.51^{a\,xy}$	0.71
1	$27.83^{a\,y}$	2.94	$20.43^{b\,x}$	0.79	$16.97^{b\,xy}$	0.66
2	$22.66^{a\,yz}$	0.38	$19.61^{a\,x}$	1.32	$20.51^{a\,x}$	1.71
3	$23.23^{a\,yz}$	1.72	$19.89^{a\,x}$	1.31	$20.13^{a\,x}$	1.93
4	$23.66^{a\,yz}$	1.23	$19.66^{b\,x}$	1.42	$19.77^{b\,x}$	0.82
5	$16.57^{a\,z}$	1.52	$18.36^{a\,x}$	1.63	$15.70^{a\,xy}$	1.07
6	$16.80^{a\,z}$	1.25	$11.63^{b\,yz}$	0.59	$16.33^{a\,xy}$	0.55
7	$16.34^{a\,z}$	1.72	$13.38^{a\,yz}$	1.43	$16.11^{a\,xy}$	0.79
8	$16.40^{a\,z}$	0.61	$15.94^{a\,xy}$	0.88	$13.26^{b\,yz}$	0.94
9	$17.11^{a\,z}$	1.50	$9.98^{b\,z}$	0.70	$10.83^{b\,z}$	0.51

^{ab}Means with the same letter are not significantly different between treatment groups $p < 0.05$.
^{xyz}Means with the same letter are not significantly different between weeks $p < 0.05$.

3 and 9, but lower at weeks 4, 5, 6, 7 and 8.

Mean blood and bone P across the entire trial had a linear relationship with DCAB, increasing as the diet became more acidiogenic, whereas mean faecal P had a curvilinear relationship with DCAB (Fig. 2), with faecal P increasing as the diet changed towards either the anionic or the cationic from the control diet, significant ($p < 0.05$) for all 3 diets. Bone P was significantly ($p < 0.05$) greater from the anionic treatment group (104.08 mg/g) compared to the cationic treatment group (100.78 mg/g) and the control group (101.39 mg/g) (Fig. 2).

The concentration of Ca in the faeces of the anionic treatment group was significantly ($p < 0.05$) greater compared to the control treatment group at weeks 1, 4, 8 and 9 (Table 1) and was significantly ($p < 0.05$) greater compared to the cationic treatment group at weeks 1, 4, 6 and 9 (Table 1). The trend was an overall decrease in bone Ca concentrations during the trial (Fig. 3). Among animals in the anionic treatment group there was a trend towards an inverse relationship between blood and bone Ca for 6 of the 9 weeks. This trend indicates a resorption of Ca from bone and its subsequent loss in the faeces as a result of the anionic diet. In addition to the acidiogenic effect, this diet also contained more Ca than the other 2 diets, 0.68 % compared to 0.53 % and 0.52 %, which could have contributed to the increased faecal Ca, but does not explain the decrease in bone Ca (Fig 3).

With the exception of weeks 1, 3 and 9 Ca and P concentrations in bone responded in opposite directions to the anionic diet (Fig. 4), and in each case the Ca:P ratio followed Ca rather than P. In the anionic treatment group the Ca:P ratio was significantly ($p < 0.05$) lower at week

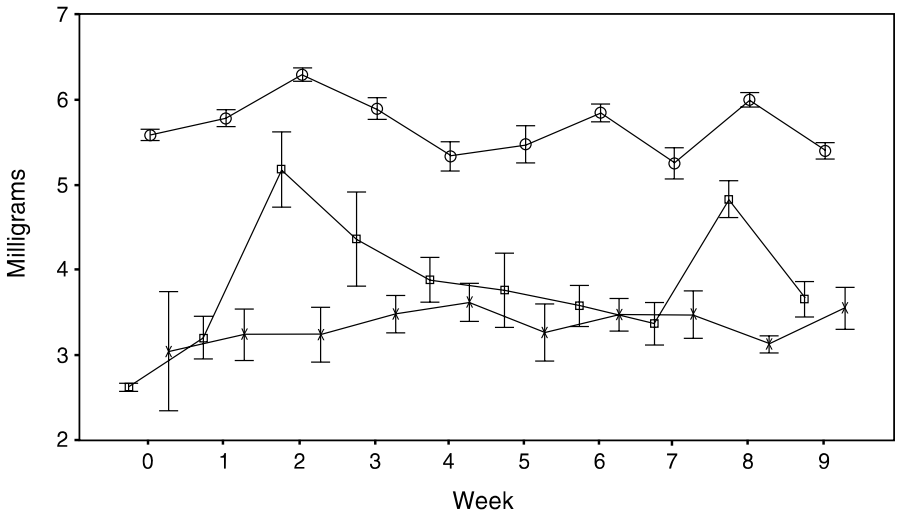

Fig. 1: **Phosphorus concentration by week, anionic diet.** ❑ **= blood** (mg% × 2); ❍ **= bone** (mg/g × 20); X **= faeces** (mg/g × 1).

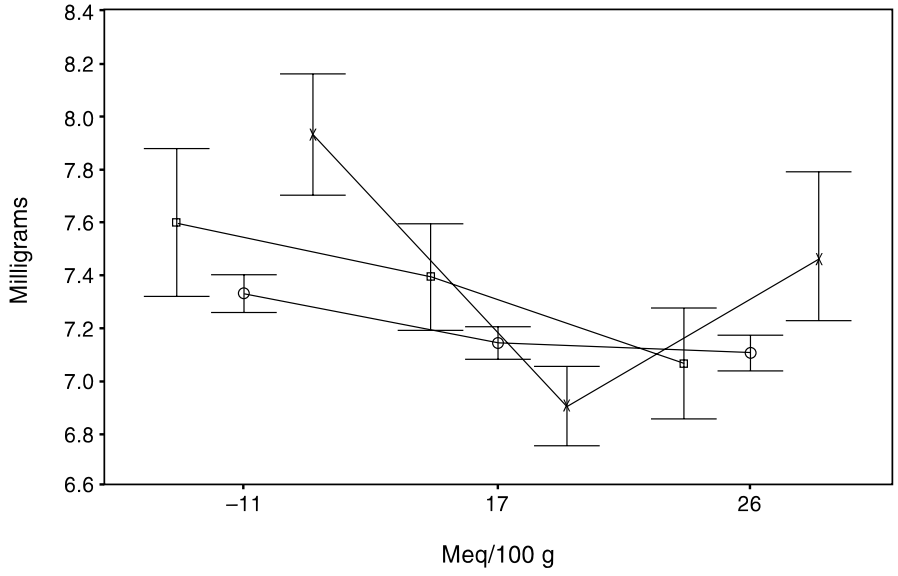

Fig. 2: **Effect of DCAB on phosphorus**. ❑ **= blood** (mg% × 1); ❍ **= bone** (mg/g × 14.2); X **= faeces** (mg/g × 0.4).

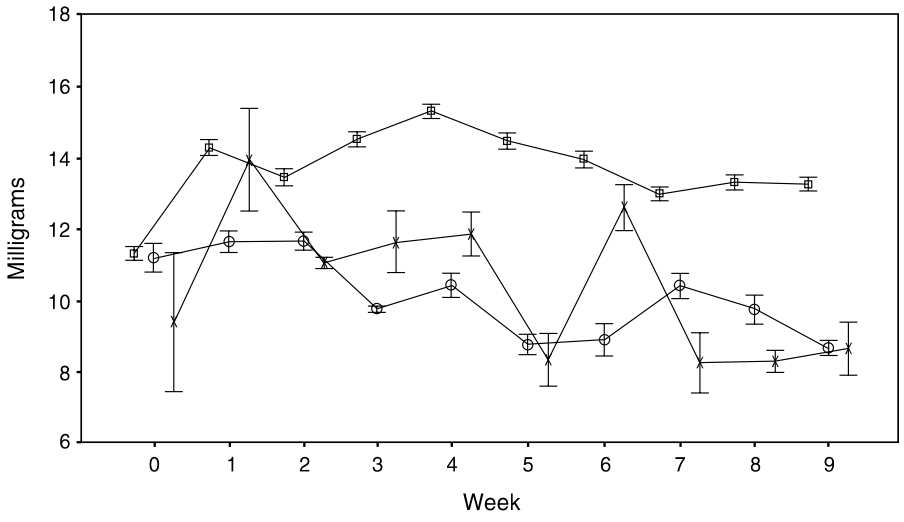

Fig. 3: **Calcium concentration by week, anionic diet**. ❑ **= blood** (mg% × 1); ❍ **= bone** (mg/g × 20); X **= faeces** (mg/g × 2).

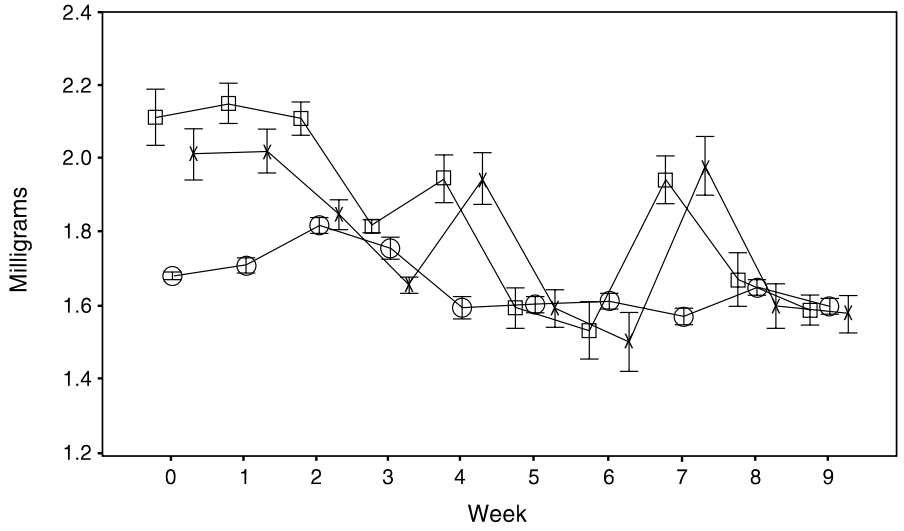

Fig. 4: **Bone calcium and phosphorus concentration in relation to CA:P ratio, anionic diet**. ❑ **= calcium** (Ca mg/g ×100); ❍ **= phosphorus** (P mg/g × 62.6); X **= Ca:P ratio** (Ca:P ratio × 1).

1 (2.02 *vs* 2.33), and significantly ($p < 0.05$) higher at week 4 (1.95 *vs* 1.55) compared to the control group, and in addition the trend was a wide variation in the Ca:P ratio among the anionic treatment group (Fig. 4). Along with peaks of 2.02 (week 1), 1.95 (week 4) and 1.99 (week 7), there were troughs of 1.66 (week 3), 1.60 (week 5), 1.51 (week 6) and 1.59 (week 9) (Fig. 4). In contrast, Teh *et al.*[13] showed that, regardless of the level of dietary P, the Ca:P ratio in the bones remained the same for all diets. What dietary P was unable to do in their experiment, high anions in the current experiment achieved.

DISCUSSION

It has been reported that an acidiogenic diet is responsible for the resorption of P from bone[2,9,15], but our results suggest not only a tendency of bone P to decrease at weeks 3, 4, 7 and 9 compared to the previous week, but also a tendency of bone P to increase among the anionic treatment group at weeks 1, 2, 5, 6 and 8 as compared to the week before, significant ($p < 0.02$) at weeks 2 and 8 (Fig. 1). Without weekly monitoring it would not have been possible to detect these acute changes in bone-mineral concentrations. Bone is traditionally considered a more or less stable tissue, with changes in mineral content occurring only over time. The results of this research alter this concept radically. Guyton[8] has described an osteolytic membrane system that allows a rapid phase of Ca and P absorption. Our results indicate that the same membrane system also allows a rapid phase of mineral resorption.

The significantly ($p < 0.05$) greater concentration of blood P in the anionic treatment group at week 2 (10.4 mg%) compared to weeks 4–6 (7.8, 7.5 and 7.2 mg%) correlates with the significantly ($p < 0.02$) less P in the faeces at week 2 (26.6 mg/g compared with weeks 4–6 (38.5, 40.1 and 38.6 mg/g) measured on an ash-weight basis. More P absorbed from the gut placed more P in the blood in the anionic treatment group. The simultaneous significantly ($p < 0.02$) higher concentration of faecal P from the anionic treatment group compared to the control treatment group at weeks 4, 6 and 8, significantly ($p < 0.05$) higher concentration of bone P at weeks 2 and 9 and significantly ($p < 0.05$) higher concentration of blood P at week 2, are all further evidence of a P-sparing effect of the anionic diet compared to the control as described by Beighle *et al.*[4]. This suggests an improvement of P utilisation due to some unknown mechanism whereby more P was made available to the animals in the anionic treatment group (Fig. 2), whereas in the control and cationic treatment groups the P was not available, possibly because it was bound to other compounds and could not be incorporated into the blood or bone, or lost in the faeces. The bone, blood and faecal P results were unexpected. Block[6] reported loss of bone Ca as the result of an anionic diet, and P was expected to respond in the same way, but mean results across the entire trial indicate a linear increase in both bone and blood P in relation to dietary cation:anion balance (Fig. 2). Our results support the hypothesis that the P homeostatic mechanisms respond differently to acidiogenic diets than those of Ca as reported by Block[6].

It has been reported[2,5] that faecal P reflects dietary P and this is in agreement with our results, as faecal P showed no significant ($p > 0.02$) difference throughout the trial within the anionic treatment group (Fig. 1). However, when faecal P values were compared among treatment groups in the present experiment, P was curvilinear in relation to DCAB (Fig. 2), with P concentrations increasing significantly ($p < 0.02$) as the diet became more acidic or more basic, but bone and blood P both reacted to DCAB with a significant ($p < 0.05$) linear increase as the diet became more acidiogenic. Despite the additional loss of P in the faeces, animals on an anionic diet still demonstrated more bone and blood P than those receiving diets that were more cationic in nature. Because of the linear increase in both blood and bone P as the diet moved from cationic to anionic, efforts should be made

to further investigate this phenomenon for possible use in formulating licks to prevent aphosphorosis.

There was a significant ($p < 0.05$) decrease in rib-bone Ca in the animals fed the anionic diet when weeks 0, 1 and 2 are compared with weeks 5, 6 and 9. The overall decrease in bone Ca across the trial and the inverse relationship between bone and blood Ca (Fig. 3) is consistent with the findings of Block[6], who suggested an increase in bone mobilisation of Ca due to an anionic diet. He reported that an anionic diet allowed for easier bone mobilisation during calcium stress. In addition in the present study, owing to the ability to monitor the more acute changes in bone tissue weekly, it was possible to detect an increase in bone Ca associated with a decrease in blood Ca at weeks 2, 6 and 7 and an increase in blood Ca associated with a decrease in bone Ca at weeks 3 and 8 (Fig. 3). This is further evidence of bone mobilisation of Ca as suggested by Block[6]. Only at weeks 1, 4, 5 and 9 did bone and blood Ca concentrations respond in the same direction (Fig. 3).

The depression of the Ca:P ratio at all sampling periods except weeks 4 and 7 (Fig. 4) was a result of the overall decrease in bone Ca during the experiment, and correlates with weeks 4 and 7, where bone Ca increased during the trial (Fig. 4).

When concentrations of bone Ca and P from the anionic treatment group were compared, only at weeks 1, 3 and 9 did these 2 minerals respond in the same direction. During the rest of the test period their response was in opposite directions. When Ca values decreased, P values increased, and when Ca concentrations rose P concentrations fell. In each case the Ca:P ratio followed the pattern of Ca (Fig. 4), in agreement with Belonje[5], who suggested that Ca had the dominant and P the subservient role in bone formation. While Ca dominated in bone formation in response to the anionic diet, P was more stable, showing less fluctuation than Ca (Fig. 4), and an overall significant ($p < 0.05$) linear increase in P concentration in bone as the diet became more acidic (Fig. 2).

The results reported here indicate a substantial effect of the anionic diet on the ability of animals to maintain a constant Ca:P ratio in the bone, with wide variations in the ratio (Fig. 4) as a result of the loss of Ca from the bone, as reported by Block[6]. More importantly, they indicate the ability of animals to retain P in the face of Ca resorption from bone as a result of an anionic diet, and despite the higher concentration of dietary Ca compared to the other 2 diets. In this trial dietary concentration of minerals, especially Ca and P, was not as important as the acidiogenic nature of the diets for the maintenance of the Ca:P ratio and bone P. Animals on the anionic diet had increasing bone P and decreasing bone Ca, except for weeks 4 and 7 (Fig. 4), despite low dietary P (0.25 %) and high dietary Ca (0.68 %).

The results indicate that an acidiogenic diet had additional effects on P homeostasis, independent of that seen in combination with Ca. Throughout the trial P acted independently of Ca, indicating that P does not follow Ca in and out of the bone, but may move independently of Ca, in agreement with the findings of Cohen[7]. Results reported here show that bone P responds more positively to dietary anions than bone Ca, with an increase in bone P and a decrease in bone Ca (Figs 1, 3).

The importance of frequency in monitoring P status in certain instances is well illustrated in this study. Previous research[2] concluded that an anionic diet resulted in resorption of P from the bone. By taking weekly samples in this trial it was demonstrated that the anionic diet was responsible not only for a decrease in the bone concentrations of P at certain stages of the experiment, but also for increased concentrations of bone P at other stages of the experiment (Fig. 1). In addition to causing bone P resorption, the anionic diet caused absorption of P by bone, and may also have led to a P-sparing effect based on results reported here of simultaneous increases in blood and bone P at weeks 2 and 8 (Fig. 1), simultaneous increases in bone and faecal P as a result of the anionic diet (Fig. 1), a linear increase in both blood and bone P in relation to DCAB (Fig. 2), and simultaneous increases in blood, bone and faecal P across the entire trial in the animals offered the diet containing –11.1 meq/100 g (Fig. 2). These results, in addition to being a further indication of a P-sparing effect of the anionic diet, also possibly explain the ameliorative effects of an anionic diet in combination with low dietary (0.22 %) P previously reported[1]. The findings of this study and the importance of bone as a reservoir of stored minerals, especially Ca and P, indicate that further investigation is required into the possible use of acidiogenic licks, and their P-sparing effect, in P-deficient animals.

The results reported here relative to the failure of the anionic diet to remove P from bone at certain stages, and at the same time the movement of Ca from bone with a concurrent increase in blood Ca due to the anionic diet (Figs 1, 3), support findings by Horst and Jorgensen[9] that ruminants fed ammonium chloride had a reduced Ca retention but no change in P retention. Our results extend the concept and suggest an increase in P retention.

When concentrations of P in blood, bone and faeces from the anionic treatment group are compared, the blood P is seen to fluctuate more than bone P or faecal P (Fig. 1), confirming findings of Cohen[7], who found bone to be a better indicator of P status than blood.

It is concluded that the P homeostatic mechanisms respond differently to an anionic diet compared to those of Ca, resulting in increases in both bone and blood P. The addition of anions could have beneficial effects when formulating licks for cattle grazing on P-deficient veld, to assist the animals in retaining the small amounts of P they are receiving. Further research is required into the formulation of anionic licks to take advantage of the P-sparing effects of anions in the diet.

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REFERENCES

1. Beighle D E, Tucker W B, Hemken R W 1988 Interactions of dietary cation-anion balance and phosphorus: effects on growth and serum inorganic phosphorus in dairy calves. *Journal of Dairy Science* 71: 3362–3368
2. Beighle D E, Tucker W B, Hemken R W 1990 Interactions of dietary cation:anion balance and phosphorus: effects on blood, bone and faecal phosphorus concentration in dairy calves. *Journal of the South African Veterinary Association* 62: 5–8
3. Beighle D E, Boyazoglu P A, Hemken R W 1993 Use of bovine rib bone in serial sampling. *Journal of Dairy Science* 76: 1047–1052
4. Beighle D E, Boyazoglu P A, Hemken R W 1995 Short term effects of dietary cation: anion balance on bone mineral homeostasis in the bovine. *Journal of the South African Veterinary Association* 66: 56–60
5. Belonje P C 1978 An investigation into possible methods of assessing the intake of calcium and phosphorus by grazing sheep. *Onderstepoort Journal of Veterinary Research* 45: 7–22
6. Block B 1984 Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. *Journal of Dairy Science* 67: 2939–2948
7. Cohen R D H 1973 Phosphorus nutrition of beef cattle 2. Relation of pasture to phosphorus content of blood, hair and bone of grazing steers. *Australian Journal of Experimental Agriculture and Animal Husbandry* 13: 5–8

8. Guyton A G 1981 *Medical physiology* (6th edn). W B Saunders, Philadelphia
9. Horst R L, Jorgensen N A 1974 Effect of ammonium chloride on nitrogen and mineral balance in lactating and nonlactating goats. *Journal of Dairy Science* 57: 683–688
10. Jardon P W 1995 Using urine pH to monitor anionic salt programs. *Compendium on Continuing Education* 17: 860–862
11. Read M V P, Engels E A N, Smith W A 1986 Phosphorus and the grazing ruminant. 2. The effects of supplementary P on cattle at Glen and Armoedsvlakte. *South African Journal of Animal Science* 16: 7–12
12. SAS *User's Guide: Statistics* 1982 SAS Institute, Cary
13. Teh T H, Hemken R W, Bull L S 1982 Evaluation of urea ammonium polyphosphate as a phosphorus source for dairy calves. *Journal of Animal Science* 55: 174–179
14. Theiler A, Viljoen P R, Green H H, Du Toit P J, Meier H 1920 The cause and prevention of lamziekte. *Journal of the Department of Agriculture* 1: 221–244
15. Vagg M J, Payne J M 1970 The effect of ammonium chloride induced acidosis on calcium metabolism in ruminants. *British Veterinary Journal* 126: 531–537

Book review — Boekresensie

Manual of standards for diagnostic tests and vaccines (3rd edn)

1996. Office International des Épizooties, 723 pp. Price: FrF 800, US$ 160. ISBN 92 9044 423 1.

The second edition of this manual, which appeared in 1992, has now been updated and its size and scope have increased considerably. The manual gives guidelines for diagnostic tests and vaccine production for diseases of livestock, birds, lagomorphs and bees that appear on the Office International des Épizooties, Animal Health Code List A (highly infectious diseases with high economic impact) and B (less infectious diseases but of economic and zoonotic importance). Some unlisted diseases of emerging importance in international trade (*e.g.* malignant catarrh, scrapie, leishmaniasis) have been included in the new edition.

The chapters on specific diseases have been written by experts designated by OIE as leaders in their field. Each of these chapters is preceded by a short summary of the chapter contents, and followed by details of diagnostic tests, requirements for diagnostic reagents and vaccines for the causative infectious agent. Diagnostic tests prescribed for international trade by the OIE Animal Health Code are given as well as alternative tests. The methods, advantages and disadvantages of the various tests, and cut-off values, are given. The 1996 edition includes an important introductory chapter on the validation of diagnostic tests.

For those diseases for which vaccines are available, the production process is outlined from the master seed handling to potency testing of the finished product. Introductory chapters on sterility testing, good manufacturing practice, quality control and the general principles of veterinary vaccine production are useful adjuncts to the information on specific vaccines.

It is worth noting that in some cases standards laid down by the OIE committee have been revised since the 1992 issue was published. For example, the OIE's requirement for the safety testing of foot-and-mouth disease vaccine is now in line with the stringent standards of the European Pharmacopoeia. However, the chapter on trichomonosis (*Tritrichomonas foetus* infection), which states incorrectly that no vaccines are yet available commercially, is slightly out of date

Diagnostic laboratories, regulatory authorities, vaccine producers or distributors, and possibly veterinarians involved with import or export of animals will find the 1996 issue of this OIE manual a useful reference document.

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