INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
22–0207/2022/28–3–161–166
DOI: 10.17957/IJAB/15.1965
http://www.fspublishers.org

Full Length Article

Impact of Heartwater Vaccination on Mineral Homeostasis in Friesian Calves as Reflected in Bone, Faecal and Blood Phosphorus, Calcium and Magnesium

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Received 14 May 2022; Accepted 26 July 2022; Published 23 September 2022

Abstract

There is scanty of published studies on the impact of heartwater (*Ehrlichia ruminantium*) vaccination on feacal, bone and blood mineral concentrations of Frisian calves reared in South Africa. This investigation attempts to determine the influence of heartwater vaccination on phosphorus, calcium and magnesium status of bone, faecal and blood in Friesian calves reared in South Africa. The study was performed at the large ruminant unit of North-West University, Mafikeng Campus, South Africa, where the average annual rainfall is 390 mm. Sixteen dairy Friesian calves weighing between 100 and 300 kg were allotted to into two groups designated treated and control groups. Experimental calves were given water and roughage composed of 50% lucerne and 50% buffalo grass ad libitum, and the study lasted for 6 weeks. Calves in the treated group given heartwater vaccine intravenously, whereas those in the control group were not given heartwater vaccine and served as the control. Faecal, blood and bone samples were collected from both groups and analysed statistically. Calves in both groups had similar ($P > 0.05$) feacal and bone phosphorus and magnesium concentrations. Similarly, heartwater vaccine had no effect ($P > 0.05$) on blood phosphorus levels in calves. However, the reverse was the case for conical bone thickness. Calves on heartwater vaccination had lower ($P < 0.05$) bone calcium concentration than calves in control group. In contrast, calves in control and treated groups had similar ($P > 0.05$) feacal calcium concentrations. In conclusion, calcium contents of bone were influenced by heartwater vaccination, indicating that calcium supplementation should be given to calves during heartwater vaccination.

Keywords: Friesian calves; Heartwater; Vaccination; Mineral homeostasis

Introduction

In South Africa, the livestock industry accounts for about 50% of the country's annual agricultural gross output, with cattle serving as a source of meat and income (Nowers *et al*. 2013). The rise in human population has led an increase in the demand for meat and dairy products, calling for improvements in cattle productivity (Terkawi *et al*. 2011; Dogliotti *et al*. 2014). However, the adverse effect of heartwater, one of such tick-borne diseases of ruminants have been reported (Harrus and Baneth 2005; Estrada-Pena and Venzal 2007; Dinkisa 2018). The disease is caused *Ehrilichia ruminantium* (Howell 1978), formerly called *Cowdria ruminantium*. It is spread by predominant tick specie called *Amblyomma hebraeum* (Bezuidenhout *et al*. 1994). The disease is prevalent in South Africa and the Caribbean (Casas and Carcavallo 1995; Allsopp *et al*. 2004).

Stock losses due to heartwater are high in sub-Saharan Africa and it is estimated that over 150 million animals are at risk in the region (Mukhebi *et al*. 1999; Minjauw and McLeod 2003). The disease is endemic to north-eastern parts of South Africa (Allsopp *et al*. 2004; Spickett *et al*. 2011). Infected animals experience loss of weight, high fever, nervous signs, hydropericardium, hydrothorax and oedema of the lungs and brain and death. Typically, infected ruminants experienced lower fertility, produces less milk, and has damaged hides (Mukhebi *et al*. 1999). The mortality rate from heartwater was estimated at 50% for cattle and 90% for small ruminants (Barbet *et al*. 2001). Research has shown that vaccination against heartwater is more effective in cattle than the use of acaricides and antibiotics (Allsopp 2009; Dinkisa 2018; Leask and Bath 2020). However, this method is ineffective in small ruminants because they are

To cite this paper: Modisaojang-Mojanaga MMC, IP Ogbuewu, BG Mokolopi, CA Mbajiorgu (2022). Impact of heartwater vaccination on mineral homeostasis in Friesian calves as reflected in bone, faecal and blood phosphorus, calcium and magnesium. *Intl J Agric Biol* 28:161–166

extremely prone to heartwater infections (Mahan *et al*. 2001). Prophylactic antibiotics treatment is widely used, but is quite costly, and the logistics are overwhelming when there is large number of animals to be treated and there is also the risk of antimicrobial resistance. The use of acaricide in tick control in ruminants is condemned due to its link to decreased immunity, environmental pollution and ticks developing resistance to acaricide (Allsopp and Allsopp 2007; Allsopp 2009).

Despite extensive research on heartwater control using infection and treatment method of vaccination, little or no research has been done to ascertain the effect of heartwater vaccination on mineral homeostasis mechanisms of cattle. The current study aimed to investigate the effect heartwater vaccination on blood, bone and faecal concentrations of phosphorus, calcium and magnesium in Frisian calves reared in South Africa.

Materials and Methods

This study was the research project executed for an M.Sc. degree by the first author awarded by North-West University, Mafikeng Campus. The project was conducted at the North-West University Experimental Farm (Molelwane) Mafikeng (25.81S and 25.51E), South Africa. The University farm is situated about 24 km southwest of Ramatlabama village which is the border town between South Africa and Botswana. The mean annual rainfall around the study area is 380–400 mm. The annual temperature ranged between 22–34°C during summer (December to February) and 2–16ºC during the winter (June to August).

Sixteen dairy Friesian calves weighing between 100 and 300 kg were assigned into two groups designated treated and control on weight equalization basis, with each group consisting of eight calves. All calves were orally dewormed using albendazole at 10 mg/kg body weight. Experimental calves were offered water and roughage composed of 50% lucerne (*Medicago sativa*) and 50% buffalo grass (*Bouteloua dactyloides*) *ad libitum*. Calves in treated group was vaccinated with live heartwater (*Ehrlichia ruminantium*) vaccine obtained from fresh blood of infected sheep at the dosage of 1 mL and administered intravenously, whereas those in control group did not receive the vaccine and served as the control.

Rectal temperatures for both groups were monitored and recorded twice daily. Faecal, blood and bone samples for mineral analyses were collected on weekly basis for 6 weeks from both groups. Faeces were taken from the rectum with plastic gloves and transferred to aluminum plates with each plate identified with the calf ear tag number and date. They dried for 3–7 days before being milled in a Mini Lab Planetary Ball Mill (Model No.: XQM-2A) to pass through a 2 mm sieve and thereafter transferred to clean plastic jars labeled with corresponding ear tag numbers and dates and stored for later digestion and laboratory analysis.

For bone samples collection, the animals were anesthetized with lignocaine injections BP 2% given at a rate of 5 mL per animal. To prevent infection at the biopsy site, the animals were operated on aseptically. Cortical bone was taken from the rib region following the procedures described Dixon *et al*. (2019). Thickness of the cortical bone was measured with a caliper and recorded in millimeters.

The experimental calves were restrained once a week between 7.00 and 9.00 a.m. and 3 mL of blood was taken from the jugular vein of each calf as described by Yawa *et al*. (2021) and later transferred into calibrated tubes without anticoagulant. The calves were restrained to minimize variation in blood mineral concentration. Thereafter, the blood samples were transported to laboratory in ice block container within 3 h of being collected. The serum was carefully removed, kept in cryotubes and frozen immediately at -20°C for the mineral analysis.

Feacal and bone samples were digested following the methods Beighle *et al*. (1990) and the phosphorus content determined by a standard method (Fiske and Subarrow 1925). The samples were also analysed for calcium and magnesium as described by Kaplan and Szabo (1979). Serum was analyzed for phosphorus contents as described by Fiske and Subarrow (1925).

Statistical analysis

Statistical analysis was executed in Minitab Data Analysis Software Release 7.2. Analysis of variance was done to determine whether the vaccination of the calves against heartwater influenced phosphorus, calcium and magnesium content of blood, bone and faeces. Means where significant, were separated using least significant differences (LSD). The weekly plots of the effect of heartwater vaccination on mean feacal, bone and blood mineral concentration, bone thickness, and rectal temperatures of calve were performed with Microsoft excel 2010.

Results

Results of the effect of heartwater vaccination on mean faecal phosphorus, calcium and magnesium concentrations are presented in Table 1. The mean faecal phosphorus, calcium and magnesium levels in treatment group were lower than in control group, although the differences were not significant. The weekly feacal phosphorus values of calves in the control group was higher than the treated group at weeks 1 and 2 and tended to decline at weeks 3 to 4 and later increased at week 5 (Fig. 1a). Calves in both treatment groups followed the same pattern throughout the duration of study (Fig. 1b, c). Results of the effect of heartwater vaccination on mean bone phosphorus, calcium and magnesium concentrations are presented in Table 2. Mean bone phosphorus and magnesium concentrations were much higher in the treated group than in the controls, though the

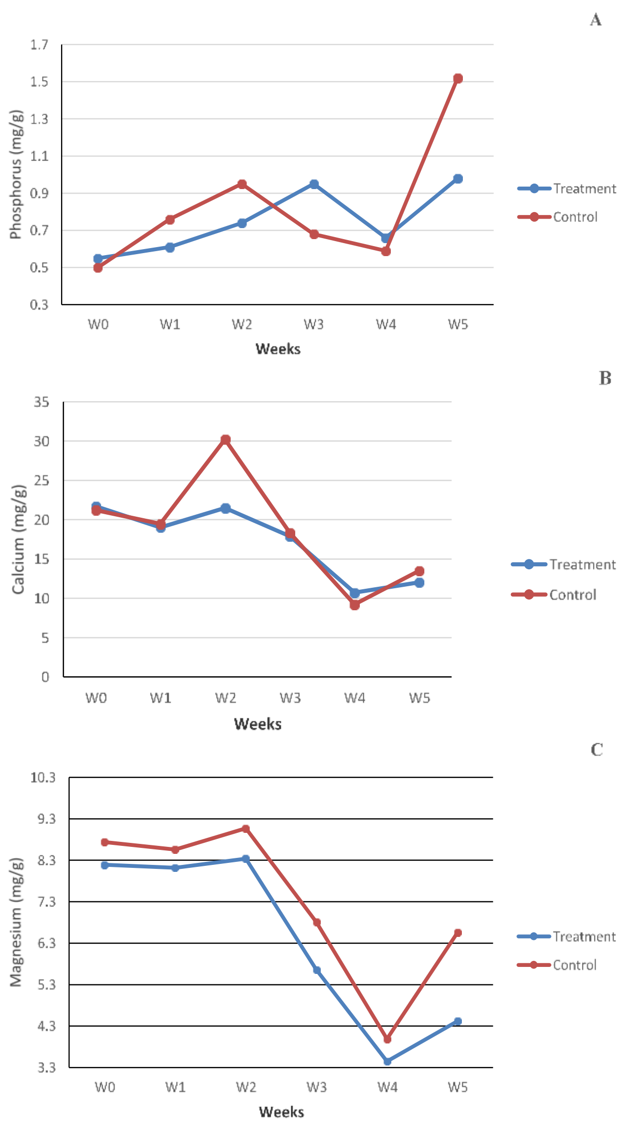

Fig. 1: Mean weekly feacal **a**) phosphorus, **b**) calcium and **c**) magnesium concentrations of Friesian calves on heartwater vaccination

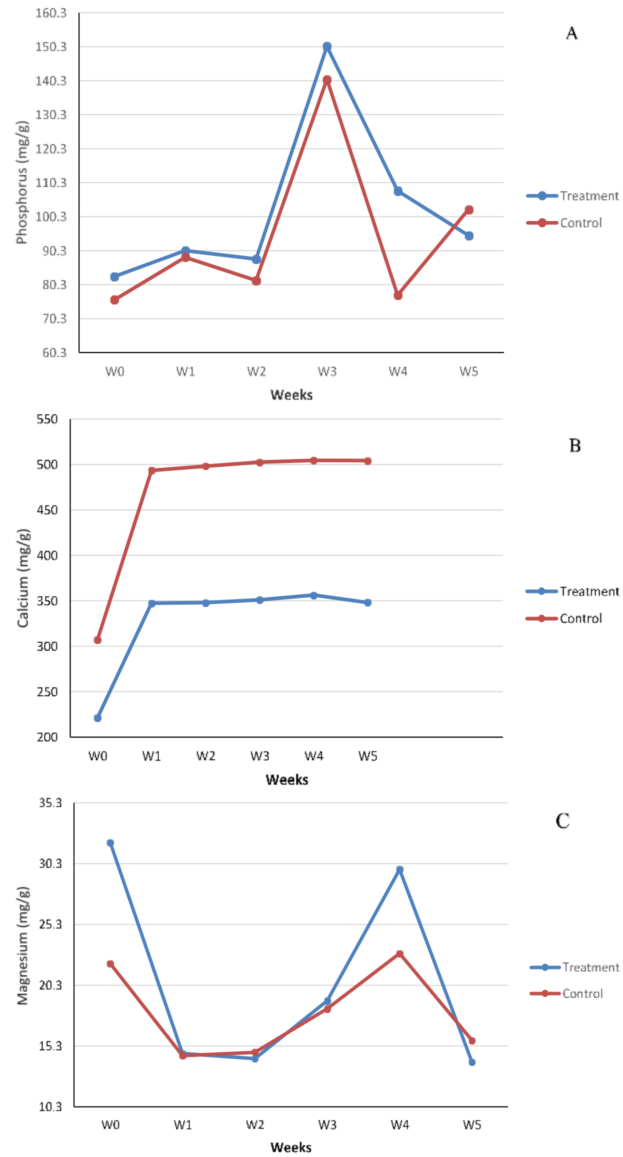

Fig. 2: Mean weekly bone **a**) phosphorus, **b**) calcium and **c**) magnesium concentrations (mg/g dry weight) of Friesian calves on heartwater vaccination

differences were not significant at $P > 0.05$. In contrast, mean bone calcium concentrations were significantly ($P < 0.05$) lower in the treated group than in the controls. There were no significant difference between the treated (1.66 ± 0.06 mg %) and the control groups (1.61 ± 0.05 mg %) in terms of blood phosphorus content. Mean blood phosphorus concentrations as presented in Table 3 showed that animals in treated group had higher blood phosphorus than the control group, although the difference was not significant ($P > 0.05$). The mean weekly bone phosphorus values of calves that received heartwater vaccine were numerically higher than calves not given the vaccine at weeks 0, 1, 2, 3 and 4 (Fig. 2a). However, the reverse was the case at week 5. In addition, calves in both treatment groups maintained a similar trend (Fig. 2b, c).

Table 2 showed that mean cortical bone thickness of calves given heartwater vaccine differed significantly lower ($P < 0.05$) from the control calves. Weekly mean thickness measured in millimeters by weeks is shown in Fig. 3 and 4. In this study cortical bone is statistically significant ($P < 0.05$) and thinner at the pre-treatment values (week 0). There was significant ($P < 0.05$) difference at week 3, when compared with the values in the controls. Cortical bone thickness tended to decrease in the treated group at week 4, although higher than the controls, but differed significantly ($P < 0.05$) at week 3 and 4. The cortical bone from the treated group was thicker ($P < 0.05$) compared to the control group at weeks 3 and 5, and tended to be thicker at weeks 1 and 4 among

Table 1: Effect of heartwater vaccination on mean feacal mineral contents of calves

Parameters (mg/g)	Control group	Treatment group	*P*-value
Phosphorus	$0.83^a \pm 0.15$	$0.73^a \pm 0.08$	0.543
Calcium	$18.67^a \pm 2.92$	$17.14^a \pm 1.90$	0.672
Magnesium	$7.20^a \pm 0.86$	$6.36^a \pm 0.88$	0.513

Means ± SEM in the same row with the same letters are not significant at $P < 0.05$

Table 2: Impact of heartwater vaccination on mean bone mineral values and thickness of calves

Parameters (mg/g)	Control group	Treatment group	*P*-value
Phosphorus	$94.33^a \pm 10.09$	$102.31^a \pm 10.23$	0.591
Calcium	$468.25^a \pm 32.30$	$328.68^b \pm 21.52$	0.005
Magnesium	$18.07^a \pm 1.51$	$20.49^a \pm 3.37$	0.527
Thickness (mm)	$1.50^b \pm 0.11$	$1.66^a \pm 0.08$	0.047

[a,b] Means ± SEM in the same row differed significantly at $P < 0.05$

Table 3: Effect of heartwater vaccination on mean blood phosphorus values of calves

Parameters (mg %)	Control group	Treatment group	*P*-value
Phosphorus	$1.61^a \pm 0.05$	$1.66^a \pm 0.06$	0.522

Means ± SEM in the same row with the same letter is not significant at $P < 0.05$

Table 4: Influence of heartwater vaccination on mean rectal temperature of calves

Parameters	Control group (°C)	Treatment group (°C)	*P*-value
Morning	$38.60^a \pm 0.11$	$38.60^a \pm 0.13$	0.542
Afternoon	$39.30^a \pm 0.07$	$39.30^a \pm 0.11$	0.415

Means ± SEM in the same row with the same alphabets are not significant at $P < 0.05$

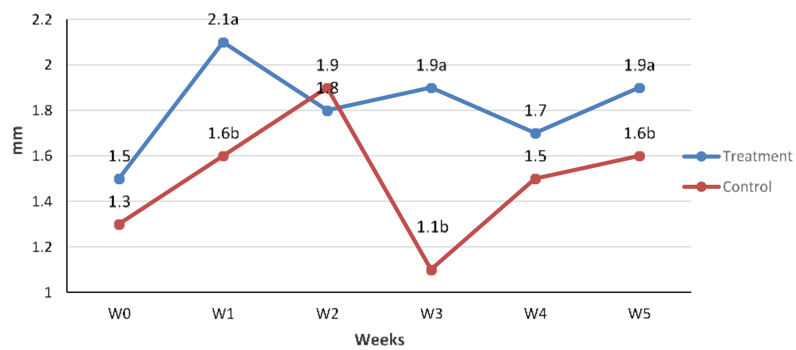

Fig. 3: Mean weekly cortical bone thickness of Friesian calves on heartwater vaccination
[a,b] Means differed significantly at $P < 0.05$

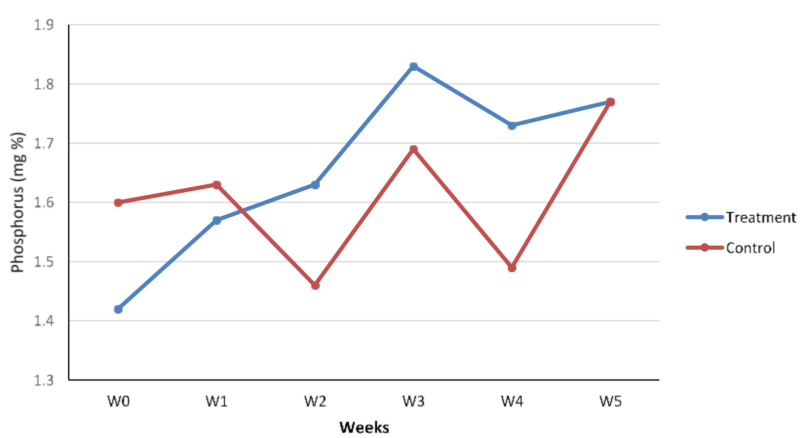

Fig. 4: Mean weekly blood phosphorus concentrations (mg%) of calves on heartwater vaccination

the treatment animals compared to controls though it was not significant ($P < 0.05$). Cortical bone samples taken after vaccination were non-significantly thicker at weeks 1, 2 and 4 in the treated group. Mean morning and afternoon rectal temperatures are shown in Table 4. There were no significant differences ($P > 0.05$) in the mean morning and afternoon rectal temperatures between the treatment groups.

Discussion

Minerals are vital nutrients in animal diets and they play a variety of role in metabolic, enzymatic and biochemical reactions that are required for sustenance, growth and development of animals. In fact, not all the minerals taken by an animal are effectively utilised, while the majority forming complex with other minerals or dietary components and then being removed as undigested feed materials. In the present research, the feacal phosphorus, magnesium and calcium recorded in the treatment group was the same as in the control group, suggesting that heartwater vaccination had no adverse effect on feacal phosphorus, magnesium and calcium content in calves. Furthermore, faecal phosphorus value of 0.73 to 0.83 mg/g obtained in this research was lower than the value of 1.6 to 4.4 mg/g reported in cattle by Beighle (2000). The observed disparity could be attributed to the effect of the vaccine on mineral uptake and utilisation as well as the amount of mineral contained in the diet which has been reported to influence feacal mineral concentration (Power and Horgan 2007). The results show that weekly feacal phosphorus, magnesium and calcium concentrations did not follow the same trend. In addition, the weekly phosphorus concentrations of calves in the control and treated groups did not maintained a consistent pattern. Contrary to the above findings, weekly feacal magnesium in the two groups followed the same trend, likewise feacal calcium concentrations. The results also revealed that feacal magnesium and calcium concentrations were lowest at week 4 post-treatment compared to week 0 (pre-treatment). Although it was not known how the vaccine reduced feacal magnesium and calcium concentrations at week 4 post-vaccination. The observed decrease in feacal magnesium and calcium at week 4 post-treatment however, could be due to vaccine's effect on the animal's body. Therefore, additional studies are required to ascertain the possible physiological reasons for the low feacal magnesium and calcium in calves at week 4 post vaccination.

Our results show that heartwater vaccination is capable of increasing bone phosphorus in calves while at the same time decreasing bone calcium. This indicates that calves receiving heartwater vaccination may require may require mineral supplementation, particularly calcium, to prevent calcium deficiencies. In contrast, heartwater vaccination had no effect on bone magnesium values in calves. Blood is used as a marker of health status in an organism (Otto *et al*. 2000). Mean blood phosphorus concentrations were much higher in the treated group than in the controls, though they are not statistically significant. The non-significant higher mean blood phosphorus values in the treated groups shows that the vaccine had less effect on blood phosphorus levels. This confirms that the phosphorus content of bone was a more reliable estimate phosphorus status than blood especially when using the developed technique by Little (1972) for collecting rib-bone sample biopsy. The results of the present study suggest heartwater vaccination tended to increase bone thickness as a result of probable more preservation of phosphorus in the bone. Mean morning and afternoon rectal temperature values recorded in this investigation were below the temperatures of above 39.5°C (morning) and 40.0°C (afternoon) considered in calves as the first sign of a heartwater reaction (Merwe 1987), suggesting that heartwater vaccination had no effect on rectal temperatures in calves. The non-significant increase in the mean afternoon rectal temperature compared to the mean morning rectal temperature control could be attributed to the fact that temperature in the afternoon tended to be higher than temperature in the morning, most likely due to hot weather conditions of the day as this study was conducted during the summer.

Conclusion

The results from this investigation indicate that calves in treated group had higher blood and bone phosphorus content than the control, but the opposite was the case for feacal phosphorus content, indicating that less phosphorus was being lost in the faeces and more was retained in the blood and bone. The findings also suggest that heartwater vaccination reduced feacal and bone calcium concentrations in calves. Heartwater vaccination caused the animals to retain more magnesium in their bone, while excreting more in the faeces.

Acknowledgements

The authors would like to express their gratitude to North West University for allowing us to use their equipment and facilitues.

Author Contributions

MMCM and BGM conceived the study, IPO and CAM statistically analysed the data, MMCM, IPO and BGM wrote the manuscript. The final manuscript was read and approved by the authors.

Conflict of Interest

The auhors declare that they have no conflict of interest to declare.

Data Availability

All data are fully available as figures and tables without restriction.

Ethics Approval

The animal study was reviewed and approved by North West University Ethics Committee for the use of live animals in research, ethics reference number: 2019/NWU_AREC/104

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