

PLASMA TOTAL CALCIUM, INORGANIC PHOSPHATE AND MAGNESIUM IN COWS AROUND CALVING IN MOROGORO, TANZANIA.

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SUMMARY

This investigation was conducted in 115 cows of second and above parity to provide baseline information on plasma levels for total calcium, inorganic phosphate and magnesium around parturition in the tropical environment. Breeds studied were Tanzania Shorthorn Zebu (TSZ), crosses of TSZ with exotic dairy breeds, and Ayrshire cattle. All animals were kept under traditional pastoral management system. Prepartal blood samples were randomly collected within the last week prior to expected calving and the postpartal blood samples were collected from the same herds within 24 hours after calving. About 51% of the indigenous TSZ and their crosses with dairy breeds had less than 2.00 mmol/l prepartal plasma calcium concentrations compared to 14.3% of Ayrshires which had 2.48 ± 0.51 mmol/l. Postpartal plasma calcium concentration was less than 2.00 mmol/l in about 65% of TSZ and their crosses, as opposed to 50% of in Ayrshires. In TSZ and their crosses plasma calcium did not drop postpartum. This drop in Ayrshires was probably due to colostrum draw of calcium linked to the relatively high milk production reported in dairy cows (14 liters/cow per day), as compared to 0.5 - 3 and 4 - 6 liters/cow/day for TSZ or their crosses, respectively. Mean prepartal plasma inorganic phosphate concentration in all breeds was relatively low compared to normal, varying from 1.11 ± 0.33 to 1.44 ± 0.34 mmol/l, between farms. An insignificant change was observed postpartum in TSZ and their crosses whereas an unexplained increase postpartum was observed in the Ayrshires. Mean prepartal plasma magnesium was significantly ($P < 0.01$) higher than normal in all breeds, with farm variation ranging from 1.15 ± 0.33 to 1.35 ± 0.56 mmol/l, and with an insignificant change postpartum.

This study supports the view that zebu cows and their crosses, are at minimum risk of developing milk fever. In contrast, Ayrshire breed kept in the same area might experience a drop in total plasma calcium at calving and could be prone to periparturient hypocalcaemia like cows of dairy breeds elsewhere.

INTRODUCTION

The dairy industry is currently gaining popularity in the developing countries. In Tanzania the driving force is to alleviate poverty in rural population through selling of milk as well as to protect environment through reduction of animal numbers. Incidences of milk fever, retained placenta and downer cow syndrome have increased during the last decade probably due to genetic improvement (Fyumagwa, 1998 unpublished).

Calcium, phosphorus and magnesium are important components of micronutrients in animals and are required for the normal functioning of a wide variety of tissues and physiological processes (Reinhardt et al., 1988). In dairy cows the subnormal plasma levels of these minerals causes detrimental effects (Radostits et al., 1999). Hypocalcaemia has been associated with low reproductive performance due to its effects on blood flow in organs including the ovaries and by its effect on gastrointestinal function (Jonsson et al., 1997). A number of periparturient diseases have also been linked with hypocalcaemia as a direct cause leading to what is termed periparturient diseases complex (Markusfeld, 1985) including uterine prolapse, left displacement of abomasum (LDA), ketosis, retained placenta, delayed uterine involution and rumen stasis.

Seasonal fluctuations in concentration of various nutrients has been reported

in tropical pastures thus, nutritional deficiency is a common problem in the dry season when nutritive value of pastures is low (van Soest, 1994). Pregnant cows during the dry season are likely to suffer more due to increased metabolic demands and in particular parturient cows are known to be vulnerable to reduced milk production and loss of weight.

There is no credible published literature available on the metabolic disease situation in dairy cattle in Tanzania. To our knowledge metabolic diseases have never been a serious problem previously, except for solitary cases in large dairy farms and small scale dairying in urban and peri-urban farming systems. Recent changes in land tenure system and agricultural policy that requires reduction of the number of indigenous cattle to protect environment has resulted into increase in number of improved breeds of cattle with higher production potential that are vulnerable to production related health problems. The dairy industry has therefore gained momentum over the last decade and veterinarians are currently needed to address diseases inherent to increased production of the dairy cow.

This study was conducted to obtain exploratory baseline information on the plasma concentration of total calcium, inorganic phosphorus and magnesium in milk producing breeds during the periparturient period.

MATERIALS AND METHODS

Study Farms

Five farms (1 – 5) were purposively chosen on the basis of accessibility by road, breeds of cattle kept, the management system in force and herd size. All farms were within a radius of 50 km from the Sokoine University of Agriculture, Morogoro Tanzania. Total numbers of animals in study farms were, farm 1 (386), farm 2 (480), farm 3 (290), farm 4 (426) and farm 5 (260) respectively.

The dominating breed in farm 1, 2 and 3 was F1 and F2 crosses of Tanzania Shorthorn Zebu (TSZ) with Friesian, Ayrshires or Jerseys. All farms practiced free-range management system and the average milk yield in these farms during previous lactation was 5 – 6 liters/cow per day. Farm 4 was dominated by TSZ, its management system was free range and the average milk yield during previous lactation ranged from 0.5 to 3 liters/cow per day. Farm 5 kept Ayrshire cattle with average milk yield of 14 liters/cow per day during previous lactation. The management system was free range but pregnant cows were offered 2 kg/day of hominy mixed with cotton seed cake at the ratio of 4 to 1 on the last week of pregnancy to meet requirements for protein and energy.

Sampling Procedure

Prepartal blood sampling was randomly done in cows within the last week before expected calving and postpartal blood sampling was done within 24 hours after calving. Blood samples were collected via the jugular vein, into evacuated test tubes containing heparin (Vacutainer[®] system). Samples were stored in the cool box during transportation to the laboratory. Plasma was obtained in the laboratory by centrifugation at 3000 rpm for 5 minutes. Plasma was frozen until analyzed for total calcium, inorganic phosphate and magnesium.

Blood Samples

A total of 115 blood samples including 74 from prepartal pregnant cows that had calved 2 times previously and 41 from postpartal cows, were randomly collected from the study farms. Of these samples, 30 were from TSZ, 15 from Ayrshire and 70 from Crosses (F1 and F2).

Analytical Procedure

Total calcium

Plasma total calcium was determined by means of a spectrophotometric analysis procedure modified by Gitelman (1967) and Kessler and Wolfman (1964). Plasma was added to acidic reagent and incubated for 4 minutes to ensure the release of protein bound calcium. An addition of an alkaline reagent formed a medium

wherein cresolphthalein complexone formed a coloured complex with calcium ions. The colour intensity is proportional to total calcium when measured at the wavelength of 574 nm.

Inorganic Phosphate

Inorganic phosphate was determined by a spectrophotometric (Unimate-kit Roche catalogue No 0736775) applied to Cobas Roche automated analyser. The analysis relies on the following reaction in an acid environment.

Inorganic Phosphorus + Ammonium Molybdate ® Phosphomolybdate Complex (blue colour). The intensity of the colour is directly proportional to the concentration of inorganic phosphate.

Magnesium

Plasma magnesium concentration was determined using the magnesium kit Magnesium procedure no. 595A (Sigma Diagnostics, ST LOUIS, MO 63178 USA). This technique is based on the following reaction with metallochromic dye calmagite: Calmagite + Magnesium ® Calmagite-Magnesium Complex (pink colour). The colour intensity is proportional to magnesium that can be measured at 520 nm.

Data analysis

Descriptive statistics (Mean and Standard deviation) of concentration for total calcium, inorganic phosphate and magnesium levels were calculated (GraphPad Prism version 2.0). Comparison was made between prepartal and postpartal concentration of minerals in each farm. Mean plasma concentrations obtained in this study were compared to reference values for plasma calcium, inorganic phosphates and magnesium given by Kaneko et al. (1997).

RESULTS

Table 1 shows the Mean (\pm SD) prepartal (within 1 week before expected calving) and postpartal (within 24 hours after calving) mean plasma concentration for total calcium, inorganic phosphate and magnesium (mmol/l) in the study farms. Cattle breeds were F1 and F2 for farm 1 –3, Tanzania Shorthorn Zebu, farm 4 and Ayrshires, Farm 5.

Table 1: Mean plasma concentration for total calcium, inorganic phosphate and magnesium (mmol/l) in the study farms.

Farm	Total calcium		Inorganic Phosphate		Magnesium	
	Prepartum	Postpartum	Prepartum	Postpartum	Prepartum	Postpartum
1.	1.95 ± 0.24	1.95 ± 0.20	1.36 ± 0.29	1.38 ± 0.36	1.33 ± 0.13	1.31 ± 0.14
2.	1.94 ± 0.33	1.96 ± 0.52	1.11 ± 0.33	1.38 ± 0.46	1.35 ± 0.56	1.26 ± 0.16
3.	2.07 ± 0.36	1.95 ± 0.40	1.32 ± 0.24	1.42 ± 0.33	1.22 ± 0.19	1.01 ± 0.16
4.	2.05 ± 0.33	1.91 ± 0.19	1.44 ± 0.34	1.34 ± 0.35	1.15 ± 0.33	1.07 ± 0.16
5.	2.48 ± 0.51	2.11 ± 0.58	1.32 ± 0.44	1.78 ± 0.28	1.20 ± 0.45	0.97 ± 0.18

Total Calcium

As shown (Table 1) the prepartal mean (\pm SD) plasma concentration for total calcium was lower in TSZ and their crosses (Farms 1 – 4) compared to normal range of 2.43 – 3.10 mmol/l for cattle (Kaneko et al., 1997). In these breeds, the difference between prepartal and postpartal plasma total calcium concentration was not significant, whereas in Ayrshire breed (Farm 5) the mean plasma prepartal calcium concentration was within the normal range, but levels declined significantly ($P < 0.05$) postpartum. About 51% of TSZ and their crosses and 14.3% Ayrshires had prepartal

calcium concentration below 2.00 mmol/l, which in this study was considered to be on the borderline towards subclinical hypocalcaemia. Moreover, postpartal blood calcium concentration was on the borderline, below 2.00 mmol/l in about 65% and 50% of TSZ and their crosses, and Ayrshires respectively (Figure 1a and 1b).

Figure 1 shows the frequency distribution for prepartal plasma calcium concentration in cattle. Class marks (x-axis) were the average of the lower and upper class marks. Blood sampling was done within the last week before expected calving.

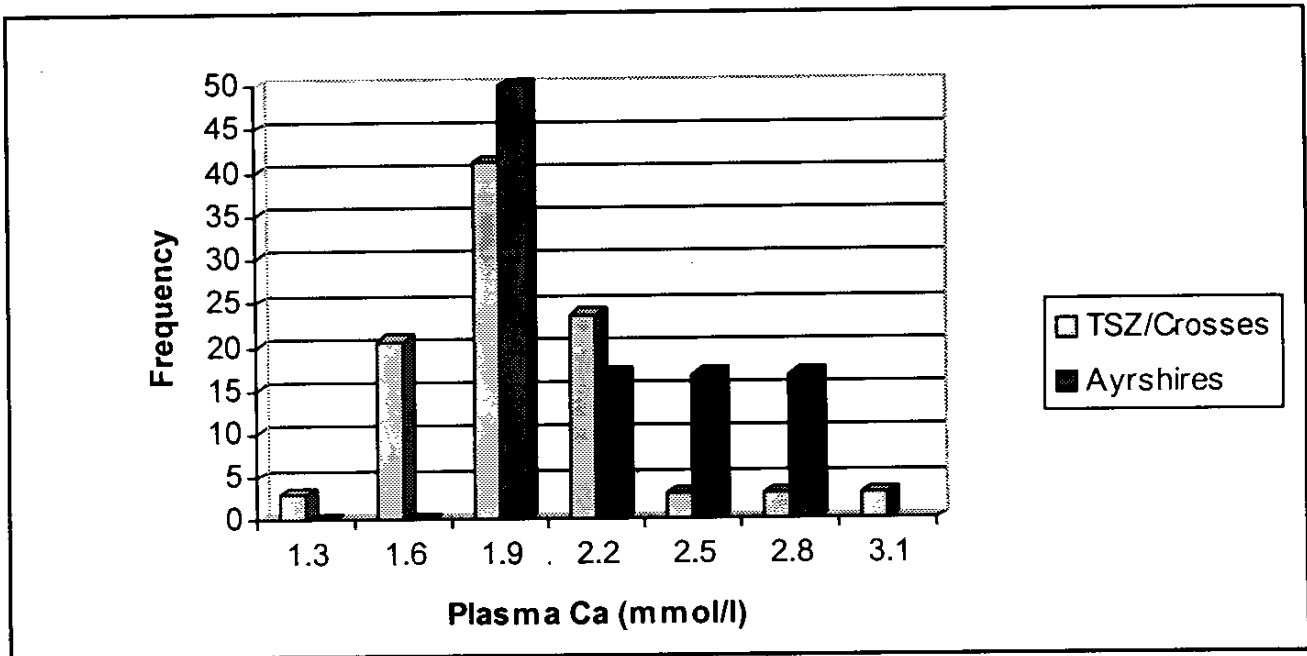


Figure 1: Prepartal plasma Calcium concentration in cattle

Figure 2 shows the frequency distribution for postpartal plasma calcium concentration in cattle. Class marks (x-axis) were the average of the

lower and upper class marks. Blood sampling was done within the last week before expected calving.

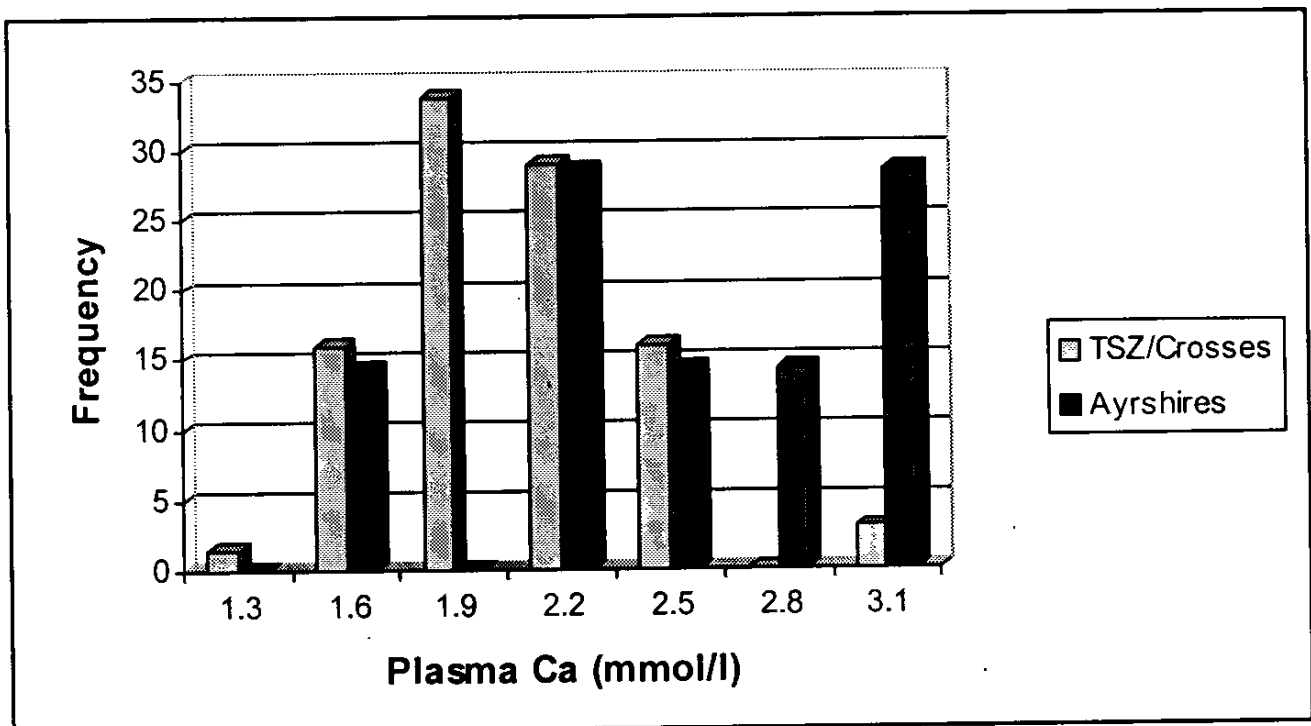


Figure 2: Postpartal plasma Calcium in Cattle

Inorganic Phosphate

As shown (Table 1) the prepartal plasma inorganic phosphate concentration in TSZ, Crosses and Ayrshires (Farm 1 – 5) were significantly lower ($P < 0.05$) than the normal range of 1.81 – 2.10 mmol/l in cattle (Kaneko et al., 1997). Except for Ayrshires where postpartal inorganic phosphate was significantly ($P < 0.05$) elevated, no significant changes were observed in inorganic phosphate levels in TSZ and their crosses.

Magnesium

The prepartal and postpartal plasma magnesium concentration for TSZ and their crosses (Farm 1 – 4) was significantly higher ($P < 0.01$) than normal values of 0.74 – 0.95 mmol/l in cattle (Kaneko et al., 1997). A slight but an insignificant postpartal drop was observed in TSZ and their crosses, whereas a significant ($P < 0.05$) postpartal drop was observed in Ayrshires (Farm 5). However, both the prepartal as well as the postpartal plasma concentrations were still higher than normal (Table 1).

DISCUSSION

In our study, blood sampling was done within 1 week before expected calving because the metabolic needs for calcium are at its maximum. Postpartal blood sampling within 24

hours after calving was decided to study the effect of colostrum drain for calcium. Also during this period any finding of subclinical levels would be a good indicator particularly in a limited survey like this.

In our study, cows expressing subclinical hypocalcaemia were defined as those in which plasma calcium concentration fell below 2.00 mmol/l, despite a wide referral range of normal plasma total calcium in cattle (Kaneko et al., 1997). This definition is similar to that previously used by others (Hove, 1986; Jonsson et al., 1999).

The management system and breed of cattle might have influenced the prepartal plasma calcium concentration in our study. Samples from TSZ and their crosses exhibited a borderline concentration for prepartal plasma calcium while Ayrshires had a normal mean prepartal calcium concentration. In TSZ and their crosses such borderline plasma concentrations of calcium might indicate insufficient dietary calcium probably due to free-range management system without additional supplementation of nutrients. This observation might be interesting because under normal physiological conditions endocrine regulated homeostatic mechanisms for calcium would have been activated and calcium levels would have been within the normal range. Whatever might be the reason for the borderline levels, low plasma levels observed in this study might be due to low dietary

calcium because the study was conducted during the dry season when the nutritive value of tropical pastures is low. These levels should however be interpreted carefully because little is known on the normal values for TSZ and their crosses kept under local conditions. In Tanzania, Mwakatundu, (1977), Pereka and Phiri, (1998) and Phiri *et al.* (1998) reported a low plasma calcium levels in dairy cattle, but grass pastures particularly in Morogoro have been reported to contain adequate calcium and phosphorus levels (Sendalo, 1988, Laffa, 1998). On the other hand, low calcium levels may reflect reduced activity of parathyroid hormone, and $1,25(\text{OH})_2\text{D}_3$ the hormones, that normally regulate calcium homeostasis. Since our study is the first of its kind in Tanzania, assaying for markers of bone metabolism in zebu and their crosses could be interesting in future studies. The higher prepartal calcium levels observed in Ayrshires compared to TSZ and their crosses could have been attributed to supplementation of hominy on the last week of gestation in farm five. Moreover, the postpartal drop in plasma calcium concentration in Ayrshires might probably be due to high (14 liters/day) milk yield in this breed compared to zebu or their crosses. Normally, high producing dairy cows experience a drop in plasma total calcium due to colostrum drain which is about 2.3g calcium per liter of colostrum (Goff and Horst, 1997).

In our study the prepartal plasma inorganic phosphate concentration was lower compared to normal referral values (Kaneko *et al.*, 1997) in TSZ, crosses as well as for Ayrshires. It was interesting in Ayrshires that postpartal plasma inorganic phosphate became significantly elevated almost to normal. These observations could not be explained due to small sample size. Under normal physiological conditions a decline in inorganic phosphate should parallel a decline in total calcium at parturition (Radostits *et al.*, 1999), although mechanisms that precipitate such a decline are not fully understood.

Both the prepartal as well as postpartal plasma magnesium concentrations were on the upper side of the normal range in all cows studied. It is possible that magnesium was adequate in pastures in the area where the study was conducted. This observation supports previous observation (Mellau, 1999, unpublished) that grass pasture magnesium levels in Morogoro ranging from 0.21 – 0.26% and were considered to be adequate. A higher postpartal plasma magnesium level is actually desired because hypomagnesemia in cattle affects absorption and metabolism of calcium and phosphorus through interference in production or secretion of PTH and activation of vitamin D to $1,25(\text{OH})_2\text{D}_3$ (Mayer and Horst, 1978). Higher postpartal plasma magnesium levels (Table 1) observed in zebu and their crosses compared to Ayrshires could be related to higher milk

production in the latter breed. A cow producing 7 liter of milk loses about 250g magnesium that is equals to an entire plasma pool in a 500 kg cow (Reinhardt *et al.*, 1988).

In conclusion, prepartal plasma calcium concentration in the majority of TSZ and their crosses in Morogoro were on the borderline, whereas inorganic phosphate levels were lower than normal. This study supports the view that zebu cows particularly TSZ and their crosses, are at minimum risk of developing milk fever probably because of their low milk production potential. In contrast, Ayrshire dairy breeds kept in the same area, might experience a drop in total plasma calcium at calving and could be prone to periparturient hypocalcaemia like other dairy cows elsewhere. However, since no literature is currently available in Tanzania regarding normal plasma mineral values in cattle the interpretation of results should be done carefully.

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