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Effect of Serum Calcium, Magnesium, and Phosphorus Levels in Cyclic and Postpartum Anestrus Jersey Cross Cows

Pema Ugyen[1], Penjor[2], and Rinzin Dorji[3]

Abstract

Mineral deficiencies and disproportion are often considered as major cause for prolonged postpartum anestrus condition in dairy cows. However, there is no clear indicator to suggest that delayed postpartum estrus is caused by inadequate mineral level for a dairy cow in Bhutan. Therefore, this study determined serum calcium, magnesium, and phosphorus levels in cyclic and postpartum anestrus Jersey cross cows and their effect on reproductive efficiency. The study was undertaken in Tsirang Dzongkhag from November to December 2013. Eighty Jersey cross cows, 40 cyclic and 40 postpartum anestrus, were sampled for the study. The mineral levels in serum samples were determined by colorimetric method in a 96 well u-bottom plate and absorbance read by microplate reader using commercial mineral kits. Data analysis was done using Statistical Packages for Social Science version 16.0. The serum calcium level was significantly higher ($p < .05$) in cyclic cows, which was estimated at 10.32 ± .21 mg/dl compared to 9.61 ± .16 mg/dl in postpartum anestrus cows. The serum magnesium levels did not vary significantly ($p > .05$) between cyclic cows and postpartum anestrus cows, which were recorded at 1.94 ± .09 mg/dl and 1.75 ± .08 mg/dl respectively. Significantly higher ($p < .05$) serum phosphorus level was recorded in cycle cows, which was 7.65 ± .14 mg/dl compared to 7.27 ± .09 mg/dl in postpartum anestrus cows. The serum calcium and phosphorus ratio was higher in cyclic cows (1.35:1) compared to postpartum anestrus cows (1.32:1). The findings indicate that low levels of calcium and phosphorus in blood can influence reproductive efficiency in dairy cows.

Key words: Cyclic cows, Jersey cross cows, postpartum anestrus, serum level

Introduction

Dairy farming is economically and socially very important component of farming system in Bhutan. It has emerged as an important source of livelihood for a majority of rural population. The increasing trend of improved cattle population and their milk production is largely due to crossbreeding of indegenous siri cattle with exotic breeds of high genetic potentials, which is considered to be a rapid and effective method of dairy cattle improvement. However, the success of dairy farming lies in proper and optimal reproductive rhythm of each individual animal with normal physical and physiological status. High milk producing dairy cattle are more vulnerable to reproductive disorder than those producing less milk due to inherent problem between good fertility and high milk production (Martinez *et al.,* 2008). Reproductive performance of dairy cow depends not only on their genetic merits, but other factors like environment, nutrition, health, and management system, which can influence reproductive efficiency (Hadush *et al.,* 2013).

Minerals play vital role in manipulation of reproductive cycles and maintenance of functional integrity of reproductive system in animals (Leathem, 1966 as reported in Niazi *et al.,* 2003). Macro minerals such as calcium, phosphorus, and magnesium have an intermediary function in the action of hormones and enzymes to initiate estrus cycle in dairy cows (Dutta *et al.,* 2001; Mahanta *et al.,* 2004). Calcium plays pivotal role in muscle contraction and

[1]Dzongkhag Livestock Sector, Punakha
[2]College of Natural Resources, Lobesa
[3]Dzongkhag Veterinary Hospital, Tsirang
Corresponding author: lingmapong@yahoo.com

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the reduction in muscle contractility is reported to decrease dry matter intake in animals (Bindari *et al.,* 2013). Deficiency and disproportion of calcium level from the set point in blood has association with anestrus condition and can affect the reproductive efficiency of animal by impairing absorption of other elements from gastro intestinal tract (Bansel *et al.,* 1978). Magnesium is considered as an activator of various enzymes transporting phosphate from adenosine triphosphate to adenosine diphosphate. Magnesium does not have direct effect on reproductive status of animals, but has close relation to calcium and phosphorus distribution and its metabolism in animals (Reddy, 2010). Phosphorus is considered to be the limiting factor hindering reproductive efficiency in dairy cows. Low phosphorus level in blood is reported to impair reproductive efficiency in dairy cows (Fraser *et al.,* 1991; Bindari *et al.,* 2013). However, information on the contribution of these minerals level in reproductive performance of dairy cow is largely unknown, especially in Bhutan.

High prevalence of postpartum anestrus and delayed estrus cycle in dairy cows could be due to low calcium, magnesium, and phosphorus levels in blood in addition to other causes. Therefore, the objective of this study was to determine the serum calcium, magnesium, and phosphorus levels in cyclic and postpartum anestrus Jersey cross cows, and to evaluate their effects on estrus cycles.

Materials and Methods

Study area

The study was undertaken at Kikhorthang, Rangthangling, and Tsholingkhar Geogs of Tsirang Dzongkhag. Dairy farming is an integral part of farming system and is one of the main sources of household income besides income from poultry rearing and orchards in the Dzongkhag. According to Livestock Statistics (2012), Tsirang Dzongkhag has 99,248 heads of livestock, of which 11,998 heads are cattle (Department of Livestock [DOL], 2012). Improved cattle, which are mainly Jersey and its crosses, account for 39% (4,679) of the total cattle population in the Dzongkhag and about 36% (1,685) of these improved cattle are found in the study area.

Selection of animals

Eighty Jersey cross cows, 40 cyclic and 40 postpartum anestrus cows, were sampled for the study. Convenience sampling method (selection of animals on availability) was used for selection of both the cyclic and postpartum anestrus cows because serum samples from cyclic cows were collected as and when the cows were brought for artificial insemination at Dzongkhag Veterinary Hospital, Tsirang. Cows which exhibited normal heat signs with no history of repeat breeding and nymphomania were considered as cyclic cows. Cows which did not show estrus signs within 90 days after calving were considered as postpartum anestrus cows. Per rectal palpation was done prior to blood sample collection to confirm their status.

Blood sampling and storage

Approximately 5 ml blood was drawn aseptically from jugular vein of each cow using sterile 18 gauge needles and collected in 10 ml vacuum tubes. Blood samples were left at room temperature for a day for serum separation. Serum that had separated as amber coloured fluid from the fibrin clot was centrifuged at 3000 rotation per minute for 5 minutes (Singh, 2002). The serum was aspirated carefully with a glass pipette into a serum vial, screw capped, labelled, and stored at -20° C in deep freezer until analysed at National Centre for Animal Health (NCAH), Serbithang.

Laboratory analysis

The serum calcium, magnesium, and phosphorus levels were determined by colorimetric method in a 96 well u-bottom plate and absorbance read by microplate reader (BIOTEK ELx800) using commercial mineral kits (QuantiChrom™ Calcium Assay Kit, QuantiChrom™ Magnesium Assay Kit, and QuantiChrom™ Phosphate Assay Kit). Standard operating procedure developed by NCAH was followed during sample analysis (NCAH, 2013). The test methods were validated by calculating coefficient of determination (R^2) using standard practice.

Computation of minerals level

The serum calcium, magnesium, and phosphorus levels of the samples were calculated as follows;

$$\text{Calcium level (mg/dl)} = \frac{\text{Value of samples - Value of buffer}}{\text{Slope}}$$

$$\text{Magnesium level (mg/dl)} = \frac{\text{Value of sample before adding EDTA - Value of sample after adding EDTA}}{\text{Value of standard before adding EDTA - Value of standard after adding EDTA}} \times 2$$

$$\text{Phosphorus level (mg/dl)} = \frac{\text{Value of sample - Value of blank}}{\text{Value of standard - Value of blank}} \times .28$$

Data analysis

Serum calcium, magnesium, and phosphorus levels between the groups were compared using independent *t*-test. Manual calculation was done to determine the ratio of the tested minerals level. The strength of association between serum mineral levels was examined using Pearson Correlation Coefficient. All statistical differences were tested at 95% confidence interval. Standard Error was used to report the variation from the sample mean.

Results and Discussion

Serum calcium, magnesium and phosphorus levels

Mean serum mineral levels of cyclic and postpartum anestrus Jersey cross cows are given in Table 1. The serum calcium level was significantly higher ($p < .05$) in cyclic cows, which was estimated at 10.32 ± .21 mg/dL compared to 9.61 ± .16 mg/dL in postpartum anestrus cows. The calcium levels in both the groups were within the normal range of 8–11 mg/dL for healthy dairy cows (Merck Sharp and Dohme Corp., 2013). The results are similar to the findings reported by Naizi *et al.* (2003) and Virmani *et al.* (2011), who recorded higher serum calcium level in cyclic cows compared to anestrus cows. The deviation of calcium level from the normal range in serum may be associated with postpartum anestrus condition in cows, which can explain that calcium may have direct influence on animal's reproductive physiology. In contrast, Dutta *et al.* (2001) reported non-significant difference on serum calcium level in cyclic and anestrus heifers. This is associated with increased number of active osteoclast in heifers, which is required for assimilation of bony tissue, giving more cells that can respond to an increased parathyroid hormone thereby increasing ability to mobilize calcium from bones when compared to older animals (Goff, 2000 as cited in Ferneborg, 2010).

Table 1. Serum mineral levels in cyclic and postpartum anestrus cows

Group	Minerals level in mg/dL (Mean ± SE)		
	Calcium	Magnesium	Phosphorus
Cyclic cows	10.32 ± .21[a]	1.94 ± .09[a]	7.65 ± .14[a]
Postpartum anestrus cows	9.61 ± .16[b]	1.75 ± .08[a]	7.27 ± .09[b]

Means with different superscripts within a column differ significantly ($p < .05$)

In this study, the serum magnesium level did not differ significantly ($p > .05$) between the two groups. The mean level of magnesium in cyclic cows was 1.94 ± .09 mg/dL when compared to 1.75 ± .08 mg/dL in postpartum anestrus cows. Although not significantly different, the magnesium level appears to be slightly higher in cyclic cows. However, the magnesium levels of both the groups were below the normal range of 1.50–2.90 mg/dL for healthy dairy cows (Merck Sharp and Dohme Corp., 2013). This indicates that the magnesium does not have direct impact on reproductive cycles of animals. Reddy (2010) stated that magnesium remains in almost hostile relation with calcium and phosphorus and any disturbance in their homeostasis can impart some influence on reproduction. Kumar (2003) also reported that reduced reproductive efficiency in cows can only encounter following general loss of appetite due to magnesium deficiency. In contrast, the current finding is not in line with the report of Kalita and Sarmah (2006), Naizi *et al.* (2003), and Dutta *et al.* (2001), who reported higher serum magnesium level in cyclic animals compared to anestrus

animals. Significantly higher ($p < .05$) serum phosphorus level was recorded in cyclic cows, which was 7.65 ± .14 mg/dL when compared to 7.27 ± .09 mg/dL in postpartum anestrus cows. The phosphorus levels in both the groups were within the normal range of 5.60–8.00 mg/dL for healthy dairy cows (Merck Sharp and Dohme Corp, 2013). Naizi *et al.* (2003) also observed higher phosphorus level in repeat breeder cows in comparison to anestrus cows. The result of this study agrees with the findings of Dutta *et al.* (2001) who reported higher serum phosphorus level in cyclic heifers compared to anestrus heifers. This indicates that low serum phosphorus level could be directly attributed to prolonged postpartum estrus in dairy cows. Seifi *et al.* (2005), Kalita and Sarmah (2006), and Jayachandran *et al.* (2013) were of similar views that phosphorus deficiency will induce lower conception rate, irregular estrus and anestrus conditions, decreased ovarian activity, and increased risk of cystic follicles in cows. Higher serum phosphorus level in cyclic cows in this study suggests that phosphorus has direct influence on the occurrence of early postpartum estrus.

Ratio of serum calcium, magnesium, and phosphorus levels

The serum calcium and phosphorus ratio was higher in cyclic cows (1.35:1) compared to postpartum anestrus cows (1.32:1) (Table 2). However, the ratio of calcium and phosphorus was lower in both the groups when compared to the normal ratio of 1.5:1 for normal dairy cows (Kumar *et al.,* 2010). The mean ratio of these minerals is regarded as utmost requirement for normal ovarian cyclicity and other reproductive rhythms of a cow. Deviation of these

mineral levels from the normal ratio may suppress absorption of other minerals from the daily feed intake. The result of this study is in line with the findings of Naizi *et al.* (2003) who reported that lower serum calcium and phosphorus ratio is the main cause for anestrus conditions in dairy cattle.

This study also found that there was low serum calcium and magnesium ratio in cyclic cows (5.31:1) compared to postpartum anestrus cows (5.49:1), but the ratios of these mineral levels were higher in both the cases in comparison to the findings of Fraser *et al*. (1991). Thus, it can be concluded that deficiency and imbalance in mineral ratio in the serum is one of the main causes of postpartum anestrus condition and it may be recommended that adequate supplementation of mineral mixture in diet is necessary to maintain the required mineral ratio in dairy cows.

Table 2. Mean ratio of serum calcium, magnesium, and phosphorus level

Minerals	Cyclic	Postpartum anestrus
Calcium : Phosphorus	1.35:1	1.32:1
Calcium : Magnesium	5.31:1	5.49:1
Phosphorus : Magnesium	3.94:1	4.15:1

Relation between serum calcium, magnesium, and phosphorus levels

Pearson Correlation Coefficient analysis (Table 3) between mineral levels in cyclic and postpartum anestrus cows indicated inverse relationship between serum calcium and phosphorus levels in cyclic cows, $r = -.41$, $p < .05$. Although not significantly related, there was also inverse relationship between serum calcium and magnesium levels in postpartum anestrus cows ($r = -.22$, $p > .05$) indicating that serum mineral levels may have been a contributing factor for prolonged postpartum anestrus condition of cows in this study. However, this result on the inverse relationship between serum calcium and phosphorus level contradicts the findings of Klimine *et al.* (2005) who reported positive correlation between serum calcium and phosphorus levels in dairy cows. In view of this, comprehensive explorations on relationship between these minerals level are required for better understanding of its contribution to cyclicity in dairy cows.

Table 1. Relation between serum calcium, magnesium and phosphorus levels

(a) Cyclic cows

Minerals	Calcium	Magnesium	Phosphorus
Calcium	1	40	40
Magnesium	.10	1	40
Phosphorus	-.41*	.09	1

* Correlation significant at the .05 levels (2-tailed)

(b) Postpartum Anestrus cows

Minerals	Calcium	Magnesium	Phosphorus
Calcium	1	40	40
Magnesium	-.22	1	40
Phosphorus	.05	.04	1

Conclusions

Deficiency or imbalance of calcium and phosphorus level prolongs the postpartum estrus in dairy cows. The present study clearly indicated higher serum calcium and phosphorus levels in cyclic cows compared to postpartum anestrus cows. Further, the result also indicated higher serum calcium and phosphorus ratio in cyclic cows than in postpartum anestrus cows. The mean ratio of these minerals level may be regarded as utmost requirement for normal ovarian cyclicity and other reproductive rhythms of a cow. Therefore, providing balanced nutritional feed and mineral supplements may improve reproductive performance of dairy cows. However, for further validation of these findings, similar study is required in different cattle breeds and agro-ecological zones, which may be prove important factors that influence the variation of serum minerals level in dairy cows.

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