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Hypomagnesemia-induced hypocalcemia: Effects on erythrocyte membrane stability in lactating jersey and Holstein-Friesian crossbreed dairy cattle

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Abstract

This study compared blood minerals, hematological parameters, and erythrocyte fragility in Jersey Cross (JX) and Holstein-Friesian Cross (HFX) cattle breeds. Significant breed differences were observed in serum calcium levels (JX: 4.34 ± 0.17 mg/dL vs. HFX: 6.26 ± 0.26 mg/dL, $p < 0.001$), serum magnesium levels (JX: 0.93 ± 0.05 mg/dL vs. HFX: 1.60 ± 0.05 mg/dL, $p < 0.001$), hemoglobin levels (JX: 4.81 ± 0.16 g/dL vs. HFX: 5.65 ± 0.25 g/dL, $p < 0.001$), mean corpuscular volume values (JX: 64.06 ± 0.98 fL vs. HFX: 75.95 ± 0.99 fL, $p < 0.001$), and erythrocyte fragility (JX: 0.10 ± 0.02 vs. HFX: 0.34 ± 0.02 , $p < 0.001$). No significant differences were observed in red blood cell count, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and packed cell volume. These findings suggest that magnesium deficiency may contribute to oxidative stress and cellular dysfunction in JX cattle, and that the erythrocyte fragility test can inform proactive strategies to counteract these effects.

Keywords: Metabolic disorders, erythrocyte fragility, oxidative stress, hematological parameters, cellular dysfunction, cross breed cattle

Introduction

Tamil Nadu's dairy sector relies heavily on crossbred cattle, particularly Holstein Friesian and Jersey breeds, to meet the increasing demand for milk production (Vijayakumar, *et al.*, 2019) [24]. However, the focus on high milk production and fat content has compromised the health and fertility of dairy cows (Hernandez CT, 2013) [7]. This has led to a surge in metabolic disorders, including milk fever, ketosis, hypomagnesemia (Mulligan and Doherty, 2008) [14]. The transition period between late pregnancy and early lactation is the most significant risk period for these disorders (Roche *et al.*, 2013). A deficiency in essential minerals like calcium, phosphorus, magnesium, or potassium can impair a cow's ability to stand, particularly postpartum. Calcium plays a crucial role in muscle contraction, and milk fever symptoms include ataxia, flaccid paresis, and recumbence (Goff J P, *et al.*, 2006) [6].

Dairy cows are prone to milk fever due to increased calcium demands during parturition and lactation. Hypocalcemia occurs when blood calcium concentrations fall below 1.87 mmol/L, with normal serum concentrations ranging from 2.0 to 2.5 mmol/L. The incidence of milk fever is around 5-7% in confined cows (Goff, 2005) [17] and 5% in grazing systems (Martín-Tereso J, *et al.*, 2014). As cows age, their ability to mobilize calcium from bone decreases, making them more susceptible to milk fever (Krehbiel, 2014). High-yielding cows are more prone to energy deficiencies and metabolic diseases due to their high energy demands (Mulligan *et al.*, 2006b) [13]. Heifers have a lower risk of milk fever than multiparous cows, but their risk increases by 9% with each additional lactation (DeGaris P, *et al.*, 2006) [10].

Magnesium (Mg) is an essential element in a cow's diet, playing a crucial role in various bodily functions (Dairy NZ, 2009) [5]. Mg is necessary for parathyroid hormone (PTH) secretion and activity, which regulates calcium homeostasis. A deficiency in Mg can lead to impaired PTH secretion and resistance to PTH receptors, reducing a cow's ability to maintain

calcium homeostasis. Mg deficiency can also lead to anemia, increased intestinal absorption of iron, decreased red blood cell counts (Sanchez-MoritoN, *et al.*, 2014) ^[21] and magnesium deficiency may be compromising the integrity of the erythrocyte membrane, making it more susceptible to oxidative damage (Lerma *et al.*, 1995) ^[11].

Cortisol measurement is a common stress indicator, but its assessment through Radioimmunoassay (RIA) and Enzyme-Linked Immunosorbent Assay (ELISA) is complex and impractical for large-scale animal welfare screening (Pawlusky *et al.*, 2017) ^[15]. Therefore, simpler evaluation indices are needed. Erythrocyte Osmotic Fragility (EOF) is a promising index, already valuable in assessing oxidative stress and transport stress in pigs (Asala *et al.*, 2011) ^[1]. EOF measures red blood cell sensitivity to osmotic changes, reflecting cell volume, membrane integrity, and surface area (Islah *et al.*, 2016) ^[8]. This study aimed to assess EOF's effectiveness in diagnosing magnesium-induced oxidative stress in Holstein-Friesian and Jersey crossbreed cattle.

Materials and Methods

This study examined milk production and hypocalcemia in 40 crossbred dairy cows (20 Holstein-Friesian and 20 Jersey) managed in village-level dairy farms in Kudimangalam block, Tiruppur district. Data collection was facilitated by veterinary officer, with on-site records including breed, parity, calving date, date of milk fever occurrence, and 305-day total milk production per cow. Clinical diagnosis of milk fever was based on distinct signs, including weakness, trembling, muscle contractions, tachycardia, hyperthermia, and tetany.

Hematological and Biochemical Analyses

Blood samples (5 ml) both whole blood and serum were collected from each of the 40 lactating dairy cows using sterile disposable syringe. Serum transferred into vacutainer tubes without anticoagulant and whole blood into EDTA added vacutainer tubes. The samples were kept at room temperature for one hours, then blood samples were centrifuged at 3000 rpm for 15 minutes, and the serum was collected in Eppendorf tubes using pasture pipettes and stored at -20 °C until analysis.

Biochemical Parameters

Selective biochemical parameters, including serum calcium and magnesium levels, were determined using commercial test kits according to the manufacturer's instructions. Serum calcium concentration was measured using the quantitative colorimetric Kit (Arkray Liquid Gold Calcium Test Kit, Arkray Pvt Ltd, Mumbai, India), while magnesium concentration was measured using the quantitative colorimetric Kit (Magnesium-L (monoTM) Calmagite Method, Reckon Diagnostic Private Ltd, Gujarat, India) using a photoelectric colorimeter (Systronics, Haryana, India) at 540 nm.

Hematological parameters

One ml of EDTA added blood used for measuring Hematological parameter such as total red blood cell count, Hemoglobin, Packed cell volume and erythrocyte indices using a hematology analyzer (Meril, Cellquant 360, India).

Erythrocyte Fragility Test (EFT)

EFT was performed as previously described by Tritschler *et al.*, (2016) ^[23]. Samples were tested within 24 hours of collection. Briefly, 20 µl of EDTA whole blood was mixed

with a series of 2 ml phosphate-buffered NaCl dilutions. After incubation at room temperature for 30 minutes, samples were centrifuged (2000 g/10 minutes), and optical densities of the supernatant of hemolysed RBC were used to detect percentage hemolysis using a photoelectric colorimeter (Systronics, Haryana, India) at 540 nm. The percentage of hemolysis in each concentration of saline was calculated assuming 100% hemolysis in distilled water.

Statistical analysis

Data were entered in Microsoft Excel 2019 and transferred to IBM SPSS (Statistical Package for Social Science) statistics 20.0 software. All data are expressed as mean±standard error of the mean (SEM). Differences were considered to be statistically significant at $p < 0.001$.

Results

Biochemical Parameters

The mean ± SE values of blood calcium and magnesium of Holstein-Friesian crossbreed and jersey crossbreed are represented in Table 1.

The serum calcium and magnesium levels showed significant breed differences between Jersey crossbreed (JX) and Holstein Friesian crossbreed (HFX) cattle. The serum calcium levels were significantly lower in JX cattle (4.34 ± 0.17 mg/dL) compared to HFX cattle (6.26 ± 0.26 mg/dL) ($p < 0.001$). Similarly, the serum magnesium levels were also significantly lower in JX cattle (0.93 ± 0.05 mg/dL) compared to HFX cattle (1.60 ± 0.04 mg/dL) ($p < 0.001$).

Hematological Parameters

The mean ± SE values of Erythrocyte indices of Holstein Friesian crossbreed and jersey crossbreed are represented in Table 2.

The Red Blood Cell Count (RBC) was slightly higher in Jersey crossbreed (JX) cattle ($4.70 \pm 0.18 \times 10^6/\mu\text{L}$) compared to Holstein-Friesian (HF) cattle ($4.65 \pm 0.17 \times 10^6/\mu\text{L}$), but the difference was not statistically significant. Both breeds showed similar Packed Cell Volume (PCV) values, with JX cattle at $21.78 \pm 1.02\%$ and HF cattle at $21.78 \pm 1.02\%$. However, a significant difference was observed in Mean Corpuscular Volume (MCV), with JX cattle having a lower value (64.06 ± 0.98 fL) compared to HF cattle (75.95 ± 0.99 fL). No significant differences were found in Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) between the two breeds. Nevertheless, a significant difference was observed in hemoglobin levels, with JX cattle having lower values (4.81 ± 0.16 g/dL) compared to HF cattle (5.65 ± 0.25 g/dL).

Erythrocyte Fragility

The mean ± SE values of Erythrocyte fragility of Holstein Friesian crossbreed and jersey crossbreed are represented in Figure 1.

The erythrocyte fragility was significantly higher in JX cattle (0.10 ± 0.02) compared to HF cattle (0.33 ± 0.02) ($p < 0.001$). This indicates that the red blood cells of JX cattle are more susceptible to hemolysis compared to those of HF cattle.

Discussion

The significant breed difference in serum calcium levels observed in this study, with JX cattle having lower levels compared to HF cattle ($p < 0.001$), is consistent with previous reports highlighting the increased susceptibility of Jersey cattle to milk fever (Chiwome, B, *et al.*, 2017) ^[3]. The lower

serum calcium levels in JX cattle may be attributed to their higher milk yield relative to their body size, as well as potential breed-specific differences in calcium metabolism (Mulligan *et al.*, 2006) [13]. This study's findings suggest that Jersey cattle may be more prone to milk fever due to lower vitamin D receptor levels (Goff, 2005) [17]. Breed-specific differences in the secretory enzyme Ca²⁺-ATPase in mammary tissue and milk fat globule membranes (Prapong *et al.*, 2005) may also contribute to the observed differences in serum calcium levels between JX and HF cattle. Additionally, factors such as age-related declines in hormone receptor numbers can impede intestinal calcium absorption, with Jersey cows having fewer 1,25-dihydroxyvitamin D₃ receptors in their intestinal mucosa compared to Holstein cows (Goff *et al.*, 2005) [17]. The significantly lower serum magnesium levels observed in JX cattle (0.93±0.05 mg/dL) compared to HF cattle (1.60±0.04 mg/dL) ($p<0.001$) have important implications for calcium metabolism and milk fever susceptibility. Hypomagnesemia, or low magnesium levels, is known to impair parathyroid hormone (PTH) secretion and its effects on target organs, leading to reduced calcium mobilization from bone (Goff *et al.*, 2005) [17]. This increases the risk of milk fever, as demonstrated by Sansom *et al.* (1983) [22], who showed that hypomagnesemia significantly impairs calcium mobilization in cows. The importance of adequate magnesium intake at parturition is underscored by a meta-analysis examining the causal factors of milk fever, which revealed that increasing dietary magnesium from 0.3% to 0.4% dry matter reduces the risk of milk fever by approximately 62% (Lean *et al.*, 2006) [10]. The significantly lower serum magnesium levels in JX cattle may contribute to their increased susceptibility to milk fever. Hypomagnesemia has a two-fold impact on calcium metabolism, reducing PTH secretion in response to hypocalcemia and decreasing tissue sensitivity to PTH. (Sansom *et al.*, 1983) [22]. The interaction between PTH and its receptor is crucial for maintaining calcium homeostasis, and hypomagnesemia can disrupt this interaction, impairing PTH's ability to act on its target tissues (Rude, 1998) [19]. The significantly lower serum magnesium levels in JX cattle may therefore impair PTH signaling and calcium mobilization, increasing their susceptibility to milk fever.

The hematological parameters of JX and HF cattle breeds were evaluated in this study, revealing significant differences in erythrocyte indices between the two breeds. The RBC count was slightly higher in JX cattle, but the difference was not statistically significant. However, a significant difference was observed in MCV, with JX cattle showing a lower value compared to HF cattle. This suggests that JX cattle may have smaller erythrocytes, which could be indicative of magnesium deficiency, as previously reported (Sanchez-Morito *et al.*, 2014) [21].

Magnesium deficiency has been shown to lead to increased intestinal absorption of iron and decreased RBC counts, possibly due to increased fragility of erythrocytes (Sanchez-Morito *et al.*, 2014) [21]. The lower MCV and hemoglobin levels observed in JX cattle in this study may be indicative of magnesium deficiency-induced alterations in erythrocyte structure and function. Additionally, magnesium deficiency has been shown to induce significant alterations in the erythrocyte membrane, including changes in fatty acid composition and fluidity (Lerma *et al.*, 1995) [11]. The significant difference in hemoglobin levels between JX and HF cattle observed in this study may also be related to magnesium deficiency-induced changes in erythropoiesis.

Magnesium deficiency has been shown to lead to increased tissue accumulation of iron, which can activate the calcium-binding protein calmodulin, disrupting the cytoskeletal structure of erythrocytes (Planells *et al.*, 1995) [11].

This study employed Erythrocyte Osmotic Fragility (EOF) as a measure of hypomagnesemia-induced oxidative stress, revealing that JX cattle exhibited higher EOF values compared to HF cattle. This indicates increased sensitivity of red blood cells to osmotic changes, suggesting that JX cattle may be more susceptible to oxidative stress due to magnesium deficiency. The use of EOF as a measure of oxidative stress is supported by previous studies, which have demonstrated that magnesium deficiency can lead to increased oxidative stress, lipid peroxidation, and protein modification (Beard *et al.*, 1977) [2]. The erythrocyte's delicate membrane structure and crucial role in oxygen delivery make it particularly vulnerable to oxidative stress. EOF measures the sensitivity of red blood cells to osmotic changes, reflecting RBC volume, membrane integrity, and surface area (Islah *et al.*, 2016) [8]. Recent studies have further validated the use of EOF as a measure of stress and physiological recovery. Reddy *et al.* (2022) [20] demonstrated that EOF could be a potential measure of stress and subsequent physiological recovery in tropical small ruminants. The higher EOF values observed in JX cattle suggest that magnesium deficiency may be compromising the integrity of the erythrocyte membrane, making it more susceptible to oxidative damage. This is consistent with previous studies, which have shown that magnesium deficiency can lead to changes in erythrocyte membrane structure and function (Lerma *et al.*, 1995) [11].

Table 1: Blood calcium and magnesium of Holstein-Friesian crossbreed and jersey crossbreed under hypomagnesemic condition

Parameter	HF cross	Jersey cross
Ca	6.26±0.26	4.34±0.17**
Mg	1.60±0.045	0.93±0.05**

Significantly different (two-tailed) from the HF Cross * $p<0.001$).

Table 2: Hematological parameters of Holstein-Friesian crossbreed and jersey crossbreed cattle under hypomagnesemic condition

Parameter	HF cross	HF cross
RBC	4.60±0.17	4.70±0.18
Hb	5.65±0.25	4.81±0.16**
MCH	24.49±0.45	23.56±0.49
MCV	75.95±0.99	64.06±0.98**
MCHC	26.62±0.53	25.77±0.62
PCV	21.78±1.02	21.78±1.016

Significantly different (two-tailed) from the HF Cross * $p<0.001$).

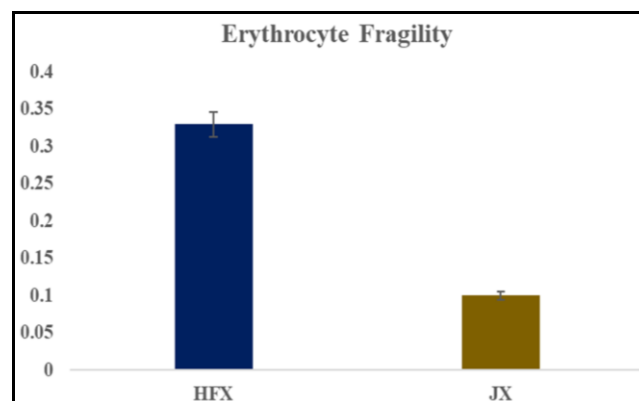


Fig 1: Erythrocyte fragility values of Holstein-Friesian crossbreed and jersey crossbreed cattle under hypomagnesemic condition

Conclusion

This study found that JX cattle are more susceptible to oxidative stress and cellular dysfunction due to magnesium deficiency. The erythrocyte fragility test can help identify individuals at risk, enabling implementation of preventive measures such as magnesium and antioxidant supplementation. This can improve the health and welfare of Jersey cross cattle, reduce disease incidence, and promote sustainable livestock production.

Conflict of Interest

Not available

Financial Support

Not available

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