


Evaluation of blood minerals and oxidative stress changing pattern in prepartum and postpartum Achai and Holstein Friesian dairy cows

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Abstract

This research focused on evaluating blood mineral levels and malondialdehyde (MDA) as an indicator of oxidative stress before and after giving birth in Achai and Holstein Friesian (HF) dairy cows. Blood samples were obtained from a total of 50 cows representing both breeds on the third, second, and first week prior to calving, as well as on the day of calving (day 0). Subsequently, samples were collected on the 1–3 weeks postpartum to allow a comprehensive evaluation of blood parameters throughout the peripartum period. Results showed a significant ($p < .01$) decrease in serum zinc (Zn) levels on the day of parturition in both breeds. Additionally, HF cows exhibited higher ($p < .01$) Zn levels on week 2 before giving birth. Interestingly, blood selenium (Se) concentration increased ($p < .01$) in HF cows on weeks 2 and 3 after calving. In contrast, Achai cows showed a rise ($p < .01$) in blood Se on week 3 of parturition. Blood copper (Cu) levels were higher ($p < .01$) on weeks 2 and 3 after parturition in HF cows and on third week before parturition compared to Achai cows, where serum Cu remained high on week 1 of parturition. The findings indicated that blood magnesium (Mg) was higher ($p < .01$) on third week before parturition in Achai cows and on weeks 2 and 3 after parturition. Serum calcium (Ca) was higher ($p < .01$) in both HF and Achai cows on weeks 2 and 3 after parturition, and lower ($p < .01$) on third week before giving birth in both breeds. The mean blood MDA levels in Achai cows were lower ($p < .01$) on weeks 1 to 3 postpartum or 3 weeks before giving birth. In HF cows, serum MDA increased ($p < .01$) just before 2 weeks of parturition and remained elevated until 3 weeks of parturition. Thus, both breeds exhibited a similar pattern of mineral fluctuations; yet, Achai cows demonstrated greater resilience to oxidative stress compared to HF cows during the transition period.

KEYWORDS

blood minerals, dairy cow, oxidative stress, parturition, pregnancy

1 | INTRODUCTION

The transition period in dairy cattle, which extends from 3 weeks prior to calving to 3 weeks post-calving, is a pivotal phase that demands careful management. This critical period encompasses a series of significant events, including re-grouping of the herd, adjustments in dietary provisions, the act of calving itself, and the onset of lactation (Macmillan et al., 2020). Within dairy cows, pregnancy initiates a cascade of crucial physiological and metabolic adaptations. These adaptations are vital to ensure the optimal development of the foetus and to provide essential substrates both during gestation and in the period following birth (Arfuso et al., 2016; Ullah et al., 2019; Ullah et al., 2023). The transition phase in dairy cows involves significant hormonal and metabolic adjustments to prepare for lactation. The cow mobilizes calcium and nutrients for milk production, increasing the risk of disorders like hypocalcaemia and ketosis. Effective management, including tailored diets and vigilant health monitoring, is vital for herd well-being and productivity. Prompt veterinary attention and regular assessments further ensure a successful lactation period (Shoukat et al., 2022).

Macro minerals play a crucial role in ensuring optimal livestock production performance by meeting the normal physiological requirements (Kurcubic et al., 2010; Sharma et al., 2006; Soetan et al., 2010). Their presence in the bloodstream holds significant importance for functions related to health, growth, reproduction, as well as the proper operation of the immune and endocrine systems. The supply and utilization of mineral-rich feed by peripheral tissues, especially the mammary gland, govern the serum levels of calcium (Ca), phosphorus (P), and magnesium (Mg) during pregnancy and lactation phases in dairy cows, providing insight into their metabolic state. If nutrient levels in dairy cows fall below the physiological threshold in dairy cows, it can lead to clinical and subclinical manifestations, adversely affecting both health and fertility (Kupczynski & Chudoba-Drozdowska, 2002; Liesegang et al., 2007; Sevinc et al., 1997; Soetan et al., 2010).

Trace minerals play pivotal roles in various enzymatic, catalytic, and structural functions in higher vertebrates (Tufarelli et al., 2016). Several environmental and biological factors can impact the concentrations of these substances in the bloodstream of mammals (Fadlalla, 2022). Numerous studies have demonstrated a decrease in serum levels of calcium (Ca), zinc (Zn), magnesium (Mg), phosphorus (P), potassium (K), and selenium (Se) during the pre-parturient period (Fadlalla, 2022; Shoukat et al., 2022; Ullah et al., 2020; Yadav et al., 2020). Furthermore, they observed that the production of colostrum, fluctuations in feed consumption, and metabolic processes during calving can have an effect on the serum levels of vitamins and trace elements. As pointed out by Shoukat et al. (2022), a rapid and significant shift in skeletal structure and mineral metabolism undergoes significant changes during the transition from pregnancy to lactation. It has been reported that cows may undergo oxidative stress during the transition

TABLE 1 Ingredients and composition of diet fed to dairy cows.

Ingredients	Dry period	Early lactation
Total mixed ration (%)		
Corn grain	25.0	28.0
Corn gluten meal	28.0	26.0
Corn seed cake	12.0	11.0
Molasses	15.0	12.0
Sunflower cake	8.0	10.0
Mustard seed cake	8.0	10.0
Dicalcium phosphate	1.0	2.0
Sodium chloride	1.0	1.0
Green grass, kg/cow/day		
Oat	20.0	30.0
Chemical analysis, %		
Crude protein	15.0	18.0
Neutral detergent fibre	26.0	32.0
Crude fat	3.4	4.3
Calcium (ppm)	0.85	1.00

period, potentially contributing to periparturient disorders that could be linked to metabolic diseases (Saqib et al., 2022; Tufarelli et al., 2023). A deficiency of trace minerals can lead to diminished animal performance, resulting in issues such as impaired reproduction, increased chances of mastitis, compromised immunity, reduced milk yield, and a higher likelihood of lameness due to laminitis (Djoković et al., 2014).

Many studies have examined minerals levels and oxidative stress in high-yielding cows during the transition phase. Yet, there is a lack of data on these factors in Achai cows compared to HF cows during this crucial period. Therefore, this study aimed to elucidate the changes in serum mineral levels and oxidative stress dynamics in Achai cows, contrasting them with HF cows throughout the transition phase.

2 | MATERIALS AND METHODS

Seventy Holstein Friesian (HF) and 50 multiparous Achai cows, averaging 5 years of age, were randomly chosen. The farm implements a 2-month dry period, with an annual milk production of about 4300 (HF) and 750 (Achai) kg, with a milk quality of 3.5% protein and 3.8% fat content (Ullah et al., 2023). Each selected cow exhibited robust health (average BCS of 3.5 ± 0.01), free from any internal or external parasites. Their pregnancy health was thoroughly monitored by expert veterinarians. All cows were provided with a carefully balanced diet designed to meet their nutritional requirements during the peripartum period and had access to a steady supply of water. Table 1 reports the chemical composition of the diets administered during the dry period and the early stages of lactation.

2.1 | Blood sampling and analysis

In the early hours, at 07:00, a meticulous procedure was followed to draw blood samples from each animal through jugular venipuncture. To maintain consistency, the same skilled operator conducted the procedure for each cow. These samples were then delicately transferred into Vacutainer tubes. Each cow underwent blood sampling at precise intervals: 3 weeks, 2 weeks, and 1 week prior to giving birth, on the actual day of calving (referred to as day 0), and subsequently on the first, second, and third weeks after calving. This comprehensive schedule allowed for a detailed assessment of blood parameters during the critical peripartum period.

After collection, the blood samples were allowed to naturally reach room temperature for a period of 20 min before being subjected to centrifugation at 3000rpm×10min. The resulting sera were then carefully stored at -20°C until the time of analysis. This rigorous process ensured the integrity and quality of the blood samples for subsequent examination of their mineral levels and oxidative stress indicators.

2.2 | Determination of serum minerals and malondialdehyde

The non-hemolysed sera underwent mineral analysis, including calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), and selenium (Se), utilizing an atomic absorption spectrophotometer (AA 6650 Shimadzu, Japan). The serum concentration of malondialdehyde (MDA) was determined following the procedure outlined by Safiullah et al. (2019). In a nutshell, a mixture comprising acetic acid, thiobarbituric acid, and sodium dodecyl sulphate was prepared. The samples were then heated for 1 h at 95°C, and centrifuged for 10 min at 3000rpm. The absorbance was measured using a spectrophotometer (IRMECO Model U2020, IRMECO QmbH, Geesthacht, Germany) at 532 nm.

2.3 | Statistical analysis

The gathered data, expressed as mean values along with their corresponding standard deviations (mean±SD), were subjected to a normality assessment using the Shapiro–Wilk's normality test. The results indicated that all the data followed a normal distribution ($p < .05$). Consequently, statistical analysis was carried out. This involved conducting a two-way ANOVA, with breed and weeks as the main factors, including their interaction. This analytical process was facilitated using statistical software (Statistix version 8.1). To identify significant differences among the means, Tukey's test was employed.

3 | RESULTS

Table 2 presents the mean blood Zn (ppm) concentration of Achai and HF cows at different intervals before and after calving. The

TABLE 2 Plasma zinc (ppm) concentration of Achai and Holstein Friesian dairy cows during the transition period.

Periods	Weeks	Achai	Holstein Friesian
Prepartum period	-2	0.58 ± 0.02 ^{ab}	0.63 ± 0.02 ^a
	-1	0.55 ± 0.01 ^{ab}	0.60 ± 0.01 ^b
Parturition day	0	0.51 ± 0.01 ^b	0.52 ± 0.01 ^b
Postpartum period	1	0.56 ± 0.01 ^{ab}	0.56 ± 0.01 ^{ab}
	2	0.53 ± 0.01 ^b	0.58 ± 0.1 ^{ab}
	3	0.56 ± 0.01 ^{ab}	0.65 ± 0.02 ^a

Note: p -values: Week=0.02; Breed=0.01; Week×Breed=0.01. Mean values with different superscripts in columns are different ($p < .05$).

TABLE 3 Blood selenium (ppm) concentration of Achai and Holstein Friesian dairy cows during the transition period.

Periods	Weeks	Achai	Holstein Friesian
Prepartum period	-2	0.29 ± 0.9 ^b	0.33 ± 0.11 ^{ab}
	-1	0.25 ± 0.07 ^c	0.30 ± 0.10 ^b
Parturition day	0	0.20 ± 0.06 ^d	0.24 ± 0.07 ^{cd}
Postpartum period	1	0.24 ± 0.07 ^{cd}	0.26 ± 0.08 ^b
	2	0.27 ± 0.08 ^b	0.35 ± 0.12 ^a
	3	0.32 ± 0.11 ^{ab}	0.39 ± 0.13 ^a
p -value		.0015	.0012

Note: p -values: Weeks=0.04; Breed=0.02; Week×Breed=0.02. Mean values with different superscripts in columns are different ($p < .05$).

results reveal a statistically significant ($p < .01$) decrease in serum Zn levels on the day of parturition for both breeds. Conversely, there is a notable increase in serum Zn levels 2 weeks of the prepartum period for HF cows, which is also statistically significant ($p < .01$).

Table 3 provides an overview of the mean blood Se (ppm) concentration in both Achai and HF cows during various days surrounding calving. It is noteworthy that HF cows displayed a significant ($p < .01$) increase in blood Se concentration on weeks 2 and 3 post-calving. On the other hand, Achai cows exhibited a significant ($p < .01$) elevation in blood Se concentration on the 21st day following parturition.

Table 4 provides an overview of the mean blood Cu (ppm) concentration in both Achai and HF cows during various days surrounding calving. Interestingly, blood Cu levels were significantly ($p < .01$) higher in HF cows on weeks 2 and 3 postparturition, as well as on week 3 prior to parturition compared to Achai cows. Notably, serum Cu remained elevated in Achai cows on week 1 following parturition. This highlights noteworthy differences in Cu levels between the two breeds during the peripartum period.

Table 5 provides a summary of the mean blood Mg (ppm) concentrations observed in both Achai and HF cows throughout the days surrounding calving. The findings indicate a significant ($p < .01$) elevation in blood Mg levels on week 3 in Achai cows, as well as on weeks 2 and 3 following parturition. This suggests notable variations

TABLE 4 Blood copper (ppm) concentration of Achai and Holstein Friesian dairy cows during the transition period.

Periods	Weeks	Achai	Holstein Friesian
Prepartum period	-2	0.28 ± 0.01 ^b	0.38 ± 0.03 ^a
	-1	0.26 ± 0.01 ^b	0.34 ± 0.03 ^{ab}
Parturition day	0	0.24 ± 0.01 ^b	0.29 ± 0.02 ^b
Postpartum period	1	0.27 ± 0.01 ^b	0.30 ± 0.03 ^b
	2	0.33 ± 0.01 ^{ab}	0.32 ± 0.02 ^{ab}
	3	0.30 ± 0.02 ^{ab}	0.38 ± 0.03 ^a

Note: *p*-values: Week=0.001; Breed=0.002; Week×Breed=0.001. Mean values with different superscripts in columns are different (*p* < .05).

TABLE 5 Blood magnesium (ppm) concentration of Achai and Holstein Friesian dairy cows during the transition period.

Periods	Weeks	Achai	Holstein Friesian
Prepartum period	-2	1.29 ± 0.02 ^a	1.19 ± 0.01 ^b
	-1	1.17 ± 0.02 ^b	1.04 ± 0.02 ^c
Parturition day	0	1.06 ± 0.01 ^d	0.96 ± 0.01 ^d
Postpartum period	1	1.19 ± 0.02 ^b	1.03 ± 0.02 ^c
	2	1.23 ± 0.02 ^b	1.23 ± 0.02 ^b
	3	1.30 ± 0.03 ^a	1.28 ± 0.03 ^a

Note: *p*-values: Week=0.001; Breed=0.002; Week×Breed=0.001. Mean values with different superscripts in columns are different (*p* < .05).

TABLE 6 Blood Ca (ppm) concentration of Achai and Holstein Friesian dairy cows during the transition period.

Periods	Weeks	Achai	Holstein Friesian
Prepartum period	-2	9.01 ± 0.02 ^b	8.99 ± 0.19 ^b
	-1	9.22 ± 0.12 ^{ab}	9.34 ± 0.18 ^a
Parturition day	0	9.12 ± 0.11 ^{ab}	9.54 ± 0.15 ^a
Postpartum period	1	9.12 ± 0.30 ^{ab}	9.53 ± 0.18 ^a
	2	10.03 ± 0.20 ^a	10.76 ± 0.23 ^a
	3	9.86 ± 0.21 ^a	9.44 ± 0.26 ^a

Note: *p*-values: Weeks=0.001; Breed=0.001; Weeks×Breed=0.001. Mean values with different superscripts in columns are different (*p* < .05).

in Mg concentrations between the two breeds during the peripartum period.

Table 6 outlines the mean blood calcium (ppm) concentrations observed in both Achai and HF cows throughout the various days surrounding calving. The results demonstrate a significant (*p* < .01) increase in serum calcium levels in both breeds on weeks 2 and 3 post-calving. Conversely, there was a significant (*p* < .01) decrease in serum calcium levels on week 3 prior to parturition in both Achai and HF cows. These findings highlight the dynamic fluctuations in

TABLE 7 Blood malonaldehyde (ng/mL) concentration of Achai and Holstein Friesian dairy cows during the transition period.

Periods	Weeks	Achai	Holstein Friesian
Prepartum period	-2	1.91 ± 0.02 ^c	2.07 ± 0.02 ^b
	-1	2.06 ± 0.02 ^b	2.30 ± 0.07 ^{ab}
Parturition day	0	2.25 ± 0.03 ^{ab}	2.56 ± 0.05 ^a
Postpartum period	1	2.10 ± 0.02 ^b	2.42 ± 0.03 ^a
	2	1.96 ± 0.03 ^c	2.34 ± 0.04 ^a
	3	1.87 ± 0.01 ^c	2.25 ± 0.02 ^a

Note: *p*-values: Week=0.001; Breed=0.002; Weeks × Breed=0.003. Mean values with different superscripts in columns are different (*p* < .05).

calcium concentrations during the peripartum period across different breeds.

Table 7 illustrates the mean blood malondialdehyde (MDA) levels in Achai and HF cows during various days surrounding calving. The results indicate a significant (*p* < .01) decrease in MDA levels in Achai cows postparturition on weeks 1, 2, and 3, as well as on week 3 before parturition. In contrast, in HF cows, serum MDA levels showed a significant (*p* < .01) increase around week 2 prior to parturition, remaining elevated up until week 3 postparturition. These observations highlight distinct MDA trends in the two breeds during the peripartum period.

4 | DISCUSSION

Macro- and micronutrients are crucial for sustaining the normal function of vital biochemical processes in a dairy cow's body. Varied degrees of deficiency in these inorganic substances can result in both clinical and subclinical symptoms, substantially diminishing the productive and reproductive performance of dairy cows (Belić et al., 2018). The peripartum phase places heightened demands on regulatory mechanisms governing the intricate milking processes (Krajnicakova et al., 2003). The study's findings demonstrate elevated serum Zinc (Zn) concentration in the prepartum period (~3 weeks), followed by a decline on the day of parturition, and a subsequent rise in the postpartum phase. Zinc plays a critical role as an essential component in over 70 enzymes involved in protein, lipid, and nucleic acid metabolism. Additionally, zinc is necessary for the normal development and functioning of the immune system. Our findings are similar to the results of Yadav et al. (2020) in crossbred cows during the peripartum period and at calving. This decline in serum Zn levels at calving is attributed to the increased production of colostrum, which redirects Zn from the plasma pool to the mammary gland. Furthermore, the reduction in Zn levels is associated with an acute phase response triggered by uterine inflammation. The stress of calving prompts the synthesis of metallothionein, a protein regulating Zn distribution, potentially aiding in scavenging hydroxide radicals and bolstering the body's inflammatory response (Prasad et al., 2004).

Consequently, Zn is redistributed from the bloodstream to tissues like the liver (Meglia et al., 2001). These combined factors account for the observed decrease in Zn levels during the peripartum period.

Copper plays a crucial role as a component in various enzymes, including antioxidant enzymes like superoxide dismutase and ceruloplasmin, as well as metabolic enzymes such as cytochrome oxidase. These enzymes contribute significantly to muscle structural integrity, detoxification of superoxide radicals, pigmentation processes, and overall energy metabolism (Djoković et al., 2014). Both Achai and HF cows showed a decline in serum Cu concentration around calving, followed by a steady increase, reaching its peak at week 3. This trend aligns with the observations of Mehere et al. (2002) and Jacob et al. (2003), who noted a gradual rise in Cu levels from the day of calving up to 4–6 weeks postpartum. The initial dip in Cu concentration at parturition, followed by a subsequent upward trend after 4–5 weeks postpartum, may be attributed to an increased transfer of this nutrient across the placenta and haemodilution during late pregnancy and at calving. Moreover, the initiation of ovarian follicular activity postpartum induces heightened circulating oestrogen levels, which, in turn, promote the binding of Cu with proteins in the liver, resulting in elevated concentrations in plasma (Mehere et al., 2002).

Selenium holds significance as a crucial micronutrient in dairy cows, serving as a key antioxidant derived from feed. It actively engages in essential enzymes and their reactions, contributing to enhanced metabolism, growth, and overall defence mechanisms within the body. This, in turn, leads to improved health, particularly in the mammary gland and reproductive system, thereby enhancing both productive and reproductive performances in animals. In our study, both Achai and HF cows exhibited a decline in serum Se levels during the prepartum period, which persisted at the time of parturition. Yet, there was a significant increase in Se concentration with each passing day postpartum for both breeds. This observation aligns with previous research by Pilarczyk et al. (2012) and Gong and Xiao (2016), where a reduction in Se concentration during the prepartum period was anticipated. This decline is expected as selenium is transferred to the foetus through the placental barrier, resulting in a lower concentration in maternal serum. As time progressed, a further decrease in blood selenium levels was anticipated, particularly at peak production, as suggested by Mikulková et al. (2020). Yet, contrary to this expectation, this study did not observe such a decline. This could perhaps be attributed to an adequate selenium content in the feed, or it might be due to the fact that the sampling period occurred too early in the postpartum period.

Magnesium serves a vital role by activating over 300 enzymes, participating in essential biosynthetic processes crucial for maintaining membrane electrical potential, and facilitating the transmission of nerve impulses (Djoković et al., 2014). A significant decrease in blood Mg levels was observed nearing parturition and in the immediate postpartum period, followed by a subsequent significant increase on weeks 2 and 3. During the peripartum phase in cows, there was a noticeable drop in Mg levels compared to the peak of lactation, indicating heightened utilization of magnesium during this critical period

(Holtenius et al., 2008). Taylor et al. (2008) proposed that levels of parathyroid hormone during calving may influence plasma magnesium concentration. While there was a decrease in calcium excretion through urine immediately after calving, it is important to note that plasma magnesium levels remained significantly lower than the intracellular level. This suggests that the observed increase in magnesium may be contingent on its movement between the intracellular and extracellular compartments. Following calving, there was a reduction in plasma magnesium levels compared to the prepartum period. Yet, postpartum levels may exhibit a slight increase, likely attributed to increased magnesium intake from the prepartum diet. It is noteworthy that other studies have also observed a reduced concentration of plasma magnesium after calving (Rérat et al., 2009).

In early lactation, there is a sudden and heightened demand for calcium, which is essential for the synthesis of milk components. Certain cows may experience a significant reduction in blood calcium levels during this period, leading to a sharp decline and subsequent development of hypocalcaemia, ultimately causing decreased neuromuscular excitability (Djoković et al., 2014). In this study, serum Ca levels exhibited a significant decrease on 3 weeks prepartum, followed by sustained elevation during the postpartum period in both breeds. The findings of Fadalla et al. (2020) similarly showed high calcium levels in early and mid-lactation, contrasting with lower levels in prepartum and postpartum stages. Patel et al. (2017) observed a distinct decline in serum calcium from early to late lactation in Kutchi camels. Coroian et al. (2017) noted that calcium levels were lowest in the initial 3 days of the colostrum period, with the highest values recorded on days 4–7, indicating an influence of lactation rank and postpartum day. These observations suggest that calcium levels may be an important factor in reproductive health risks for crossbred cows during both prepartum and postpartum periods. Shoukat et al. (2022) noted that serum calcium levels typically exhibit minor fluctuations, making it a less sensitive indicator of input/output balance. Yet, a significant reduction in calcium concentration in late pregnancy can indicate a potentially hazardous situation. In contrast, the current study found that calcium levels remained consistent from –2 weeks to 3 weeks postpartum, suggesting that cows effectively maintain stable blood calcium levels, likely through the activation of calcium reserves in their bones.

In the present trial, the levels of MDA, an indicator of oxidative stress, exhibited an upward trend shortly before parturition and continued to increase in both cow breeds. Notably, the rise was more pronounced in HF cows compared to Achai cows. The peripartum phase is marked by significant physiological adaptations to prepare for lactation commencement (Saqib et al., 2018). This period witnesses heightened metabolic activity to meet the increased energy demands associated with lactation onset. Consequently, tissues consume more oxygen as part of regular cellular respiration. This heightened metabolic activity leads to an amplified accumulation of Reactive Oxygen Species (ROS) and a suppression of the antioxidant defence system around calving (Ullah et al., 2023). The imbalance between increased ROS production and reduced antioxidant availability can result in elevated oxidative stress. The observed

increase in serum MDA levels postparturition in cows is consistent with previous studies indicating heightened oxidative stress (Gong & Xiao, 2016; Tsuchiya et al., 2020).

5 | CONCLUSION

Based on the findings of this study, it can be concluded that the transition period in dairy cows is marked by substantial alterations in mineral levels and oxidative stress. Notably, both mineral status and oxidative stress exhibited similar trends in both breeds, although the magnitude of change was significantly higher in HF cows compared to Achai cows. The assessment of MDA levels further revealed that Achai cows demonstrate greater resilience to oxidative stress compared to their HF counterparts. The observed interactions between time periods and health statuses underscore the potential of blood metabolite levels during the peripartum phase as indicators for assessing the risk of postpartum diseases. These insights offer a valuable tool for herd monitoring, presenting a fresh perspective to enhance farm management strategies.

AUTHOR CONTRIBUTIONS

All the authors equally contributed.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data that support the findings of this study are available upon request from the corresponding author.

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