



Serum Biochemical, Oxidative Stress and Cytokine Dynamics of Murrah Buffaloes during Periparturient Period

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ABSTRACT

Background: The transition period represents a critical physiological phase in dairy buffaloes, characterized by profound metabolic, biochemical, oxidative and immunological adaptations to support parturition and the onset of lactation. The present study evaluated dynamic changes in serum biochemical parameters, negative energy balance indicators, oxidative stress markers and inflammatory cytokines in murrah buffaloes during the transition period under Indian rearing conditions.

Methods: Twelve clinically healthy Murrah buffaloes were monitored from 21 days prepartum to 21 days postpartum, with blood samples collected at six defined time points (-21, -7, 0, +7, +14 and +21 days relative to calving) to study evaluated serum biochemical parameters, negative energy balance indicators, oxidative stress markers and inflammatory cytokines.

Result: Significant increases in non-esterified fatty acids (NEFA) and β -hydroxybutyrate (BHBA) concentrations were observed around calving and early postpartum, indicating pronounced negative energy balance. Concurrent declines in serum glucose, cholesterol, triglycerides and lipoproteins, along with alterations in protein fractions, liver enzyme activities and mineral levels, reflected metabolic stress and hepatic adaptation. Oxidative stress was evidenced by elevated malondialdehyde concentrations and a transient reduction in antioxidant enzyme activities during early lactation. Additionally, inflammatory cytokines showed marked modulation, with reduced pro-inflammatory markers and elevated anti-inflammatory responses at calving, suggesting temporary immunosuppression. Overall, the findings highlight the complex interplay between energy metabolism, oxidative stress and immune regulation during the transition period in Murrah buffaloes and emphasize the need for targeted nutritional and management strategies to mitigate metabolic and health disorders during early lactation.

Key words: Early lactation, Inflammatory cytokines, Murrah buffalo, Negative energy balance, Oxidative stress, Serum biochemistry, Transition period.

INTRODUCTION

Biochemical evaluations are indispensable diagnostic tools for assessing the physiological, metabolic and nutritional status of dairy animals, particularly during critical phases such as the periparturient period (Mondal and Paul, 2012; Sharma *et al.*, 2018). These assessments provide valuable insights into internal homeostasis and aid in the early detection of subclinical metabolic and infectious disorders, thereby facilitating timely preventive and therapeutic interventions to improve herd health and productivity (Singh *et al.*, 2015).

The transition period, extending from three weeks before to three weeks after parturition, is considered one of the most physiologically demanding stages in the productive life of dairy buffaloes. During this phase, animals undergo substantial endocrine, metabolic and immunological adaptations to support fetal growth, calving and the onset of lactation. Although these changes are essential for lactogenesis, they also predispose animals to metabolic stress, oxidative imbalance and immune suppression when not adequately managed (Wankhade *et al.*, 2017; Vasantha *et al.*, 2025; Mondal *et al.*, 2026).

A major challenge during the transition period is the marked increase in energy demand associated with colostrum and milk production, coupled with reduced dry

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matter intake around calving. This often results in negative energy balance (NEB), leading to excessive mobilization of body fat reserves and increased concentrations of non-esterified fatty acids (NEFA) and ketone bodies. Consequently, animals become more susceptible to metabolic disorders such as ketosis and fatty liver syndrome, along with impaired immune and reproductive functions (LeBlanc, 2010).

Buffaloes play a crucial role in the Indian dairy sector owing to their high milk fat content, adaptability and significant contribution to national milk production (FAO, 2020). However, compared to dairy cattle, relatively limited information is available regarding the metabolic and physiological adaptations of buffaloes during the transition period. Species-specific differences in nutrient utilization, thermotolerance and stress responsiveness necessitate dedicated investigations in buffaloes rather than direct extrapolation from cattle studies (Campanile *et al.*, 2010; Das *et al.*, 2016).

Blood metabolites and biomarkers including non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), glucose, proteins, liver enzymes, oxidative stress indices and inflammatory cytokines are considered reliable indicators of energy balance, hepatic function, oxidative status and immune competence during the transition period (Bionaz *et al.*, 2007). Therefore, the present study was undertaken to evaluate alterations in serum biochemical, oxidative stress and inflammatory parameters in Murrah buffaloes during the transition period under Indian rearing conditions. The findings may contribute to a better understanding of metabolic and immunological adaptations in buffaloes and support the development of improved nutritional and herd health management strategies.

MATERIALS AND METHODS

Ethical approval

All experiments were conducted as per the guidelines of committee for control and supervision of experiments on animals (CCSEA) after due approval from the Institute Animal Ethical Committee of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (Reference no. IAEC/SVPUAT/2023/133).

Experimental animals and management

The study was conducted in the year 2023-24 at the Livestock Research Farm Complex of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut. Twelve healthy pregnant Murrah buffaloes during the last trimester of gestation were selected randomly for the experiment. All animals selected were between parity three to five and were maintained under identical husbandry practices throughout the study period. The buffaloes were housed in clean and well-ventilated loose housing sheds under standard farm management conditions. Continuous access to fresh drinking water was provided. Feeding was carried out according to the routine nutritional schedule of the farm, consisting of green fodder, dry roughage and concentrate mixture formulated to meet the nutrient requirements of advanced pregnancy and early lactation. The enrolled animals had a body condition score ranging from 3-4 on a five point scale and an average milk production of 10-15 kg/day during early lactation. Prior to enrolment, all buffaloes were subjected to clinical examination and

were found free from any detectable metabolic, infectious, or reproductive abnormalities.

Blood sampling

A total of 72 blood samples were collected aseptically from the buffaloes by jugular vein puncture during the study period. Samples were collected at six time points: -21 and -7 days before calving, on the day of calving (day 0) and at +7, +14 and +21 days postpartum. Because the exact date of calving could not always be predicted, prepartum samples were collected with a permissible variation of ± 3 days.

Estimation of negative energy balance indicators

Non-esterified fatty acids (NEFA) and β -hydroxybutyric acid (BHBA) were estimated by the enzymatic colorimetric method using a commercially available ELISA kit (ImmunoTag, Cat. no. ITE050021 and ITE050267) at an optical density of 550-570 nm and 340 nm following the manufacturer's instructions.

Biochemical estimation

Serum biochemical parameters including lipid profile, liver enzymes, minerals, protein profile and renal function indices were estimated using commercially available diagnostic kits (Erba Diagnostics) according to the manufacturer's instructions. Serum concentrations of total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein (VLDL) were analyzed for lipid profiling. Liver function parameters included alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST), while mineral estimation comprised calcium (Ca), inorganic phosphorus (P), magnesium (Mg) and glucose levels. Protein profile parameters such as total protein, albumin, globulin, albumin-globulin (A:G) ratio, along with blood urea nitrogen (BUN) and plasma creatinine, were also quantified. All analyses were performed using a semi-automatic biochemistry analyzer (Erba chem 7 transasia biochemistry analyzer).

Oxidative stress markers

The concentrations of malondialdehyde (MDA) was determined using a TBARS assay-based kit (ImmunoTag, Cat. no. ITFA0025) at an optical density of 550 nm. The activity of catalase (CAT) and GSH were estimated by the enzymatic colorimetric method using a commercially available ELISA kit (ImmunoTag, Cat. no. ITFA02025 and IT11802) at an optical density of 240 nm and 410 nm respectively, whereas SOD activity was assessed using a WST-1 based colorimetric assay (Bioassay Technology Laboratory, Cat. no. SH0039) at an optical density of 450 nm following the manufacturer's instructions.

Inflammatory cytokines

The serum levels of tumor necrosis factor-alpha (TNF- α), interferon-gamma (IFN- γ) and interleukin-10 (IL-10) were

estimated using commercially available ELISA kits (ImmunoTag cat. No. ITE050019, ITE050005 and ITE050252) at an optical density of 450 nm following the manufacturer's instructions.

Statistical analysis

The data generated during the experiment were analyzed using appropriate statistical software. Results are expressed as mean $\pm$ standard error (SE). Differences among sampling periods were evaluated using repeated-measures statistical procedures and significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

During the transition period, dairy buffaloes undergo profound metabolic, biochemical and immunological changes to support the onset of lactation and restore physiological homeostasis following parturition. In the present study, the occurrence of NEB was clearly evidenced by significant ($P \leq 0.05$) alterations in circulating energy metabolites, lipid fractions, liver enzymes, minerals, oxidative stress markers and inflammatory cytokines during the transition period.

Serum non-esterified fatty acids (NEFA) and β -hydroxybutyrate (BHBA) are well-established indicators of adipose tissue mobilization and hepatic ketogenesis, respectively. In the present study, NEFA concentrations increased progressively from -21 days prepartum (294.35 ± 5.36 $\mu\text{mol/L}$) to peak levels at +7 days postpartum (412.80 ± 5.14 $\mu\text{mol/L}$), followed by a gradual decline by +21 days postpartum. Similarly, BHBA concentrations rose from 36.82 ± 1.22 nmol/mL at -21 days to a maximum at +7 days postpartum (58.95 ± 1.76 nmol/mL) before decreasing thereafter (Fig 1 a,b). These trends reflect intensified lipolysis and increased hepatic uptake of fatty acids during early lactation, when glucose availability is prioritized for lactose synthesis (Herdt, 2000; Gross *et al.*, 2011; Lisuzzo *et al.*, 2024). Comparable patterns have been reported in dairy cows and buffaloes, wherein peak NEFA and BHBA concentrations occur within the first week

postpartum, coinciding with maximal milk yield and lowest dry matter intake (LeBlanc, 2010; McArt *et al.*, 2013). Similar metabolic alterations associated with negative energy balance have also been reported in postpartum buffaloes (Sriranga *et al.*, 2023). Persistently elevated NEFA and BHBA are known to impair immune cell function, hepatic metabolism and reproductive performance, thereby increasing the risk of metabolic disorders such as ketosis and fatty liver syndrome.

In the present study significant alterations in the lipid profile were observed during the whole transition period (Table 1). Serum total cholesterol, triglycerides, HDL-C, LDL-C and VLDL concentrations declined markedly around calving, reaching their lowest values at +7 days postpartum, followed by gradual recovery by +21 days. These changes are primarily attributed to reduced hepatic synthesis of lipoproteins, increased utilization of cholesterol for steroid hormone synthesis and impaired triglyceride export from the liver during NEB (Grummer, 1995; Bionaz *et al.*, 2007). Hypocholesterolemia and hypotriglyceridemia during early lactation have been consistently reported in buffaloes and dairy cows and are considered indicators of compromised hepatic lipid metabolism (Mondal and Paul, 2012; Das *et al.*, 2016). The reduced availability of circulating lipoproteins further exacerbates hepatic fat accumulation, contributing to oxidative stress and inflammation.

Activities of liver enzymes and concentrations of minerals and glucose also changed significantly during the transition period (Table 2). Serum calcium, phosphorus and magnesium concentrations declined around calving and early postpartum, reflecting increased mineral demand for colostrum secretion, milk synthesis and neuromuscular function (Goff and Horst, 1997; Goff, 2008). Subclinical hypocalcemia during early lactation has been associated with reduced feed intake, impaired immune function and increased susceptibility to postpartum disorders. Elevated activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) observed in the present study indicate increased

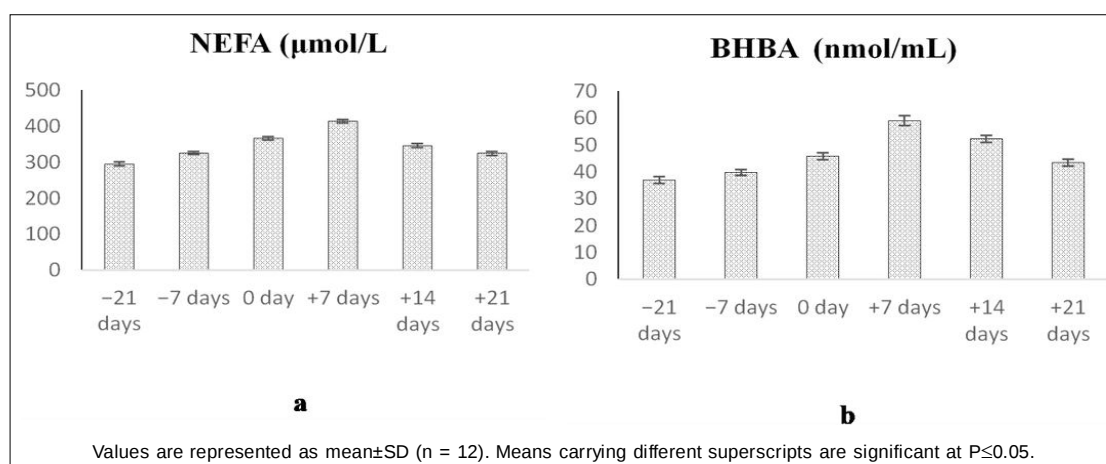


Fig 1: Dynamic changes in negative energy balance markers in murrah buffaloes during the transition period.

hepatic workload, enhanced gluconeogenesis and intensified lipid metabolism during NEB (Contreras *et al.*, 2010; Puppel *et al.*, 2015). Similar increases in hepatic enzyme activities during the transition period have been reported in buffaloes by Kour *et al.* (2024), indicating enhanced hepatic metabolic stress around parturition. Serum glucose concentrations declined at calving and remained low until +14 days postpartum, confirming the persistence of NEB, with partial recovery by +21 days as feed intake improved and metabolic adaptation progressed (Yadav *et al.*, 2013).

Total protein and globulin concentrations declined at calving, likely due to hemodilution and the selective transfer of immunoglobulins into colostrum to support passive immunity in newborn calves. Rathwa *et al.* (2023) similarly observed alterations in serum total protein and albumin concentrations during the transition period in Surti buffaloes, reflecting metabolic adaptation and hepatic functional changes. In contrast, albumin levels increased slightly, resulting in an elevated albumin-to-globulin ratio.

These findings suggest a transient redistribution of protein fractions rather than impaired hepatic protein synthesis. Blood urea nitrogen (BUN) and creatinine concentrations peaked at calving, reflecting increased protein catabolism, enhanced amino acid oxidation for gluconeogenesis and transient renal adaptation to increased metabolic load (Mondal and Paul, 2012; Mishra *et al.*, 2013; Mondal and Paul, 2012; Mishra *et al.*, 2013; Bademkiran *et al.*, 2020). Similar increases in BUN around parturition have been linked to increased mobilization of body protein reserves during NEB (Table 3).

Markers of oxidative stress exhibited pronounced changes during the transition period, reflecting a clear imbalance between reactive oxygen species (ROS) generation and antioxidant defense mechanisms in dairy buffaloes. Malondialdehyde (MDA), a well-recognized marker of lipid peroxidation, increased significantly at calving and reached peak concentrations at +7 days postpartum (Fig 2a). This elevation in MDA indicates enhanced oxidative damage to cellular and mitochondrial membranes during early lactation, a period characterized

Table 1: Dynamic changes in Lipid profile of murrah buffaloes during the transition period (n=12).

Days Parameter	-21 days	-7 days	0 day	+7 days	+14 days	+21 days
Total cholesterol (mg/dL)	98.24±2.09 ^a	96.37±1.68 ^a	88.14±2.01 ^b	83.14±1.86 ^c	89.36±1.83 ^b	98.36±2.08 ^a
Triglyceride (mg/dL)	27.61±0.86 ^a	26.82±0.72 ^a	18.28±1.06 ^b	17.14±0.96 ^b	19.21±1.23 ^b	23.14±1.13 ^c
HDL-C (mg/dL)	61.46±1.68 ^a	58.16±1.23 ^a	50.26±1.38 ^{bc}	48.21±1.89 ^{bc}	47.13±1.16 ^b	51.36±1.76 ^{bc}
LDL-C (mg/dL)	50.17±1.62 ^a	48.17±1.16 ^a	41.18±1.23 ^b	37.26±1.63 ^c	42.14±1.89 ^b	46.26±1.72 ^{da}
VLDL (mg/dL)	4.34±0.16 ^a	3.98±0.28 ^a	2.06±0.32 ^b	3.16±0.18 ^c	3.27±0.43 ^a	3.51±0.32 ^a

Mean having different superscripts in the same row differ significantly (P≤0.05).

Table 2: Dynamic changes in mineral and liver profile of murrah buffaloes during the transition period (n=12).

Days Parameter	-21 days	-7 days	0 day	+7 days	+14 days
Calcium (mg/dL)	11.16±0.44 ^a	10.11±0.13 ^{bd}	9.94±0.56 ^{cd}	9.96±0.11 ^{cd}	9.18±0.63 ^d
Phosphorus (mg/dL)	5.76±0.56 ^a	5.19±0.32 ^a	4.67±0.23 ^b	4.88±0.42 ^b	4.74±0.31 ^b
Magnesium (mg/dL)	3.18±0.08 ^a	3.35±0.11 ^a	2.95±0.12 ^b	2.14±0.23 ^b	2.83±0.21 ^b
AST (u/l)	90.44±1.41 ^a	94.56±1.51 ^b	101.79±1.67 ^c	105.56±0.24 ^d	101.84±1.08 ^c
ALT (u/l)	26.86±0.24 ^a	28.24±0.27 ^a	30.22±0.21 ^b	34.16±0.29 ^c	34.24 ±0.26 ^c
ALP (u/l)	74.45±3.63 ^a	82.84±2.76 ^b	92.27±1.78 ^c	98.39±1.19 ^d	99.13±1.76 ^d
Glucose (mg/dL)	67.64±1.22 ^a	68.61±0.48 ^a	57.69±1.31 ^b	59.69±0.92 ^b	59.46±1.79 ^b

Mean having different superscripts in the same row differ significantly (P≤0.05).

Table 3: Dynamic changes in protein profile of murrah buffaloes during the transition period (n=12).

Days Parameter	-21 days	-7 days	0 day	+7 days	+14 days	+21 days
Total protein (g/l)	7.69±0.34 ^a	7.28±0.28 ^a	6.49±0.27 ^b	7.64±0.23 ^b	7.36±0.19 ^a	7.46±0.18 ^a
Albumin (g/l)	3.54±0.18 ^a	3.46±0.15 ^a	3.62±0.13 ^b	3.78±0.16 ^b	3.53±0.12 ^a	3.55±0.14 ^a
Globulin	4.15±0.14 ^a	3.82±0.16 ^a	2.87±0.12 ^b	3.86±0.11 ^a	3.83±0.16 ^a	3.91±0.14 ^a
A:G ratio	0.85±0.08 ^a	0.91±0.09 ^a	1.26±0.08 ^b	0.98±0.06 ^a	0.92±0.04 ^a	0.91±0.03 ^a
BUN (mmol/l)	28.42±0.32 ^{ac}	27.43±0.96 ^a	32.96±0.48 ^b	29.82±0.53 ^c	28.78±1.09 ^a	27.81±0.82 ^a
Creatinine (μmol/l)	1.23±0.19 ^a	1.25±0.13 ^a	2.16±0.14 ^b	1.92±0.13 ^b	1.28±0.14 ^a	1.24±0.07 ^a

Mean having different superscript in the same row differ significantly (P≤0.05).

by intensified metabolic activity and extensive mobilization of body fat reserves. The surge in circulating non-esterified fatty acids (NEFA) during negative energy balance (NEB) increases hepatic fatty acid uptake and mitochondrial β -oxidation, thereby promoting excessive electron leakage from the respiratory chain and subsequent ROS production. Concurrently, the activities of key antioxidant enzymes, including superoxide dismutase, catalase and glutathione peroxidase, declined markedly around calving and during the immediate postpartum period (Fig 2b,c,d). This transient suppression of antioxidant capacity may be attributed to overwhelming ROS production, depletion of endogenous antioxidant reserves and reduced availability of antioxidant precursors and micronutrient cofactors under conditions of NEB (Bernabucci *et al.*, 2005; Jain and Shakkarpude, 2024; Sordillo and Aitken, 2009). Similar reductions in antioxidant enzyme activities during the periparturient period have been associated with increased susceptibility to metabolic stress and immune dysfunction. The temporal coincidence of peak MDA concentrations with elevated NEFA and β -hydroxybutyrate (BHBA) levels further supports the mechanistic link between excessive lipolysis, hepatic ketogenesis, mitochondrial overload and oxidative stress (Contreras *et al.*, 2010; Lisuzzo *et al.*, 2024). The gradual restoration of antioxidant enzyme activities by +21 days postpartum suggests an adaptive upregulation of endogenous antioxidant defense systems as metabolic balance improves, feed intake increases and oxidative pressure is alleviated (Yang *et al.*, 2018; Sharma *et al.*, 2017; Gessner *et al.*, 2021). Altered antioxidant status

during the transition period has been linked with metabolic stress and lactation-associated physiological adaptations in Murrah buffaloes (Chaudhari *et al.*, 2017). These findings highlight the importance of managing oxidative stress during the transition period to safeguard metabolic health and immune competence in dairy buffaloes.

Serum inflammatory cytokines exhibited significant modulation during the transition period, reflecting dynamic changes in immune competence and physiological adaptation. Pro-inflammatory cytokines, including TNF- α and IFN- γ , declined sharply at calving, reaching their lowest concentrations on the day of parturition (day 0), followed by a gradual increase during the early postpartum period (Fig 3a,b). This transient suppression of pro-inflammatory cytokines may represent a physiological adaptation to prevent excessive immune activation during parturition, which could otherwise damage maternal tissues. In contrast, IL-10, a key anti-inflammatory cytokine, increased at calving and gradually declined thereafter, suggesting a temporary shift toward an anti-inflammatory state that supports tissue repair and limits inflammatory damage associated with placental separation, uterine involution and tissue remodeling (Fig 3c). The observed cytokine profile indicates that parturition is accompanied by a tightly regulated immunosuppressive and anti-inflammatory phase, likely aimed at protecting the dam from excessive inflammatory responses while maintaining essential immune surveillance. The postpartum resurgence of pro-inflammatory cytokines reflects the re-establishment of immune competence and functional recovery of the maternal immune

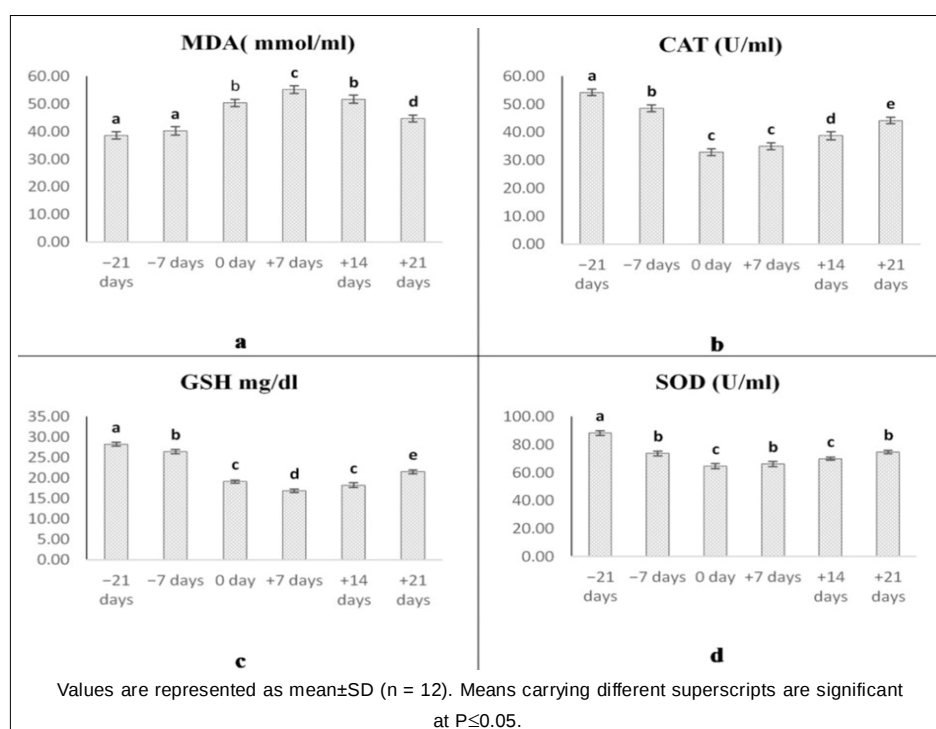


Fig 2: Dynamic changes in oxidative stress markers in murrah buffaloes during the transition period.

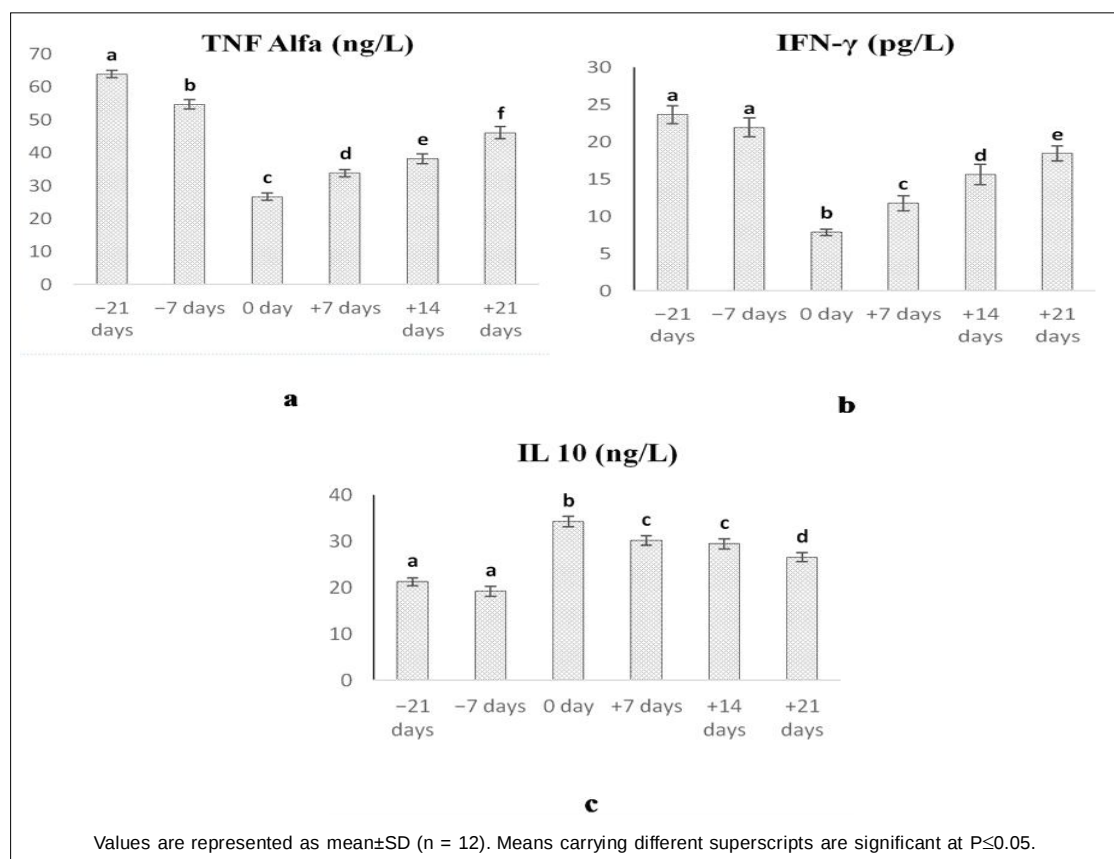


Fig 3: Dynamic changes in inflammatory cytokines in murrah buffaloes during the transition period.

system, which is crucial for protecting against infections and supporting early lactation. Similar cytokine dynamics have been reported in periparturient dairy cows and buffaloes, highlighting their close association with metabolic stress, oxidative imbalance and overall transition period adaptation (Contreras *et al.*, 2010; Gomaa *et al.*, 2021; Ciliberti *et al.*, 2025). One limitation of the present study is the relatively small number of animals included (n = 12), which may restrict the broader applicability of the findings. Nevertheless, repeated observations on the same animals throughout the transition period and uniform management conditions helped minimize biological variation. Further investigations involving larger populations are warranted to confirm the present results.

CONCLUSION

The present study demonstrates that the transition period in Murrah buffaloes is associated with significant metabolic, oxidative and immunological changes. Increased NEFA and BHBA concentrations, along with reduced glucose and lipid fractions, confirmed the occurrence of negative energy balance during early lactation. Alterations in liver enzymes, protein metabolites and mineral status reflected increased metabolic and hepatic demands, while elevated malondialdehyde levels and reduced antioxidant enzyme activities indicated heightened oxidative stress around

calving. Changes in inflammatory cytokines further suggested a transient immunosuppressive state at parturition followed by gradual postpartum immune recovery. These findings highlight the importance of appropriate transition-period management. Provision of energy-dense and balanced diets, adequate mineral and antioxidant supplementation, maintenance of optimal body condition and strategies to maximize dry matter intake can help reduce metabolic stress and support immune function. Regular monitoring of biomarkers such as NEFA, BHBA and glucose may facilitate early detection of animals at risk of transition-related disorders, thereby improving health, productivity and reproductive performance in Murrah buffaloes.

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Disclaimers

The opinions and conclusions expressed in this article are solely those of the authors and do not necessarily reflect the views of their affiliated institutions. The authors are responsible for ensuring the accuracy and completeness of the information presented; however, they do not accept any liability for any direct or indirect losses arising from the use of this content.

Informed consent

All animal experimental procedures were conducted in accordance with ethical standards and were approved by the Institutional Animal Ethics Committee (IAEC).

Conflict of interest

On behalf of all authors, I hereby state that there are no conflicts of interest related to this manuscript.

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