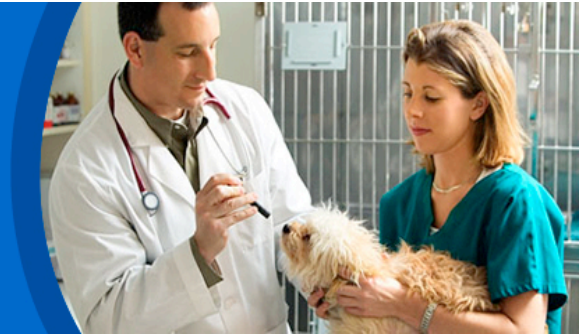




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Biochemical and hormonal profile changes during periparturient period in nili-ravi buffaloes

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Abstract

The periparturient period is the single most metabolically demanding phase in the productive life of a dairy buffalo, yet biochemical monitoring protocols for Nili-Ravi buffaloes remain poorly standardised. This research characterised changes in key serum biochemical and hormonal parameters across three reproductive stages pre-partum (-30 to -7 days), partum (day 0±2 days) and post-partum (+7 to +30 days) in 54 clinically healthy pluriparous Nili-Ravi buffaloes maintained at the Cairo Research Institute of Animal Sciences experimental farm between October 2022 and September 2023. Blood samples were collected by jugular venipuncture at weekly intervals and analysed for glucose, total protein, blood urea nitrogen (BUN), aspartate aminotransferase (AST), calcium, phosphorus, progesterone and cortisol. Glucose dropped from a pre-partum mean of 58.3±6.2 mg/dL to 42.1±5.8 mg/dL at partum ($p<0.001$) before recovering to 52.7±5.1 mg/dL post-partum. Total protein and calcium followed similar troughs at parturition (6.1±0.5 g/dL and 7.6±0.9 mg/dL, respectively). BUN and AST rose sharply around calving, peaking at 24.3±4.2 mg/dL and 89.6±11.3 U/L. Progesterone declined steeply from 6.8±1.2 ng/mL pre-partum to 1.2±0.5 ng/mL at parturition, while cortisol surged from 14.5±3.1 ng/mL to 28.7±4.2 ng/mL. By day +30, most parameters had returned toward pre-partum baselines, though calcium recovery lagged behind other analytes. These reference intervals should help field veterinarians identify buffaloes at risk of metabolic disorders during the transition period.

Keywords: Nili-Ravi buffalo, periparturient period, biochemical profile, hormonal changes, progesterone, cortisol, transition period, calcium, glucose, metabolic monitoring

1. Introduction

No phase in the dairy buffalo production cycle carries as much metabolic risk as the three weeks before and after calving. During this narrow window, energy demand for foetal growth, colostrum synthesis and uterine involution can exceed voluntary feed intake by 20-30 percent, predisposing the animal to negative energy balance, hypocalcaemia and hepatic lipidosis ^[1]. In Nili-Ravi buffaloes the dominant dairy breed across riverine buffalo-producing countries the problem is compounded by a naturally higher body condition score at dry-off, which accentuates lipolysis when appetite drops ^[2]. Biochemical profiling during the transition period provides an early-warning system. Glucose concentration reflects the balance between hepatic gluconeogenesis and peripheral glucose uptake by mammary and uterine tissues ^[3]. BUN rises when protein catabolism outpaces urea clearance. AST serves as a marker of hepatocellular stress linked to fatty acid infiltration of the liver ^[4]. Calcium, whose extracellular pool must support both skeletal homeostasis and smooth-muscle contraction during parturition, often falls below safe thresholds if intestinal absorption and bone resorption fail to compensate for colostrum losses ^[5]. Hormonal axes are equally informative. Progesterone sustains pregnancy and drops precipitously within 24 hours of luteolysis to permit parturition, while cortisol surges under the dual influence of foetal hypothalamic-pituitary-adrenal activation and maternal stress ^[6]. Both hormones interact with metabolic pathways: cortisol promotes gluconeogenesis but suppresses immune function, and the speed of progesterone decline has been linked to retain foetal membranes in buffaloes ^[7]. Although extensive transition-period data exist for Holstein-Friesian cattle, relatively few controlled investigations have tracked the full biochemical and hormonal trajectory in Nili- Ravi

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Buffaloes under Standardised conditions [8]. Most available reports are retrospective, involve mixed parities or lack serial sampling. Egypt's Nili-Ravi population, estimated at 3.5 million head, contributes over 70 percent of the country's buffalo milk output, yet reference intervals specific to this breed and climate remain incomplete [9]. The present research aimed to establish stage-specific reference ranges for seven biochemical analytes and two hormones across the pre-partum, partum and post-partum stages in clinically healthy Nili-Ravi buffaloes, and to test whether the magnitude of periparturient change differed among analytes.

Material and Methods

Material

Fifty-four clinically healthy pluriparous Nili-Ravi buffaloes (parity 2-5, age 5-9 years, body weight 480-620 kg) were selected from the experimental herd of the Cairo Research Institute of Animal Sciences, Giza Governorate, and Egypt. Animals had no history of metabolic or reproductive disorders in the preceding lactation and were maintained under a semi-intensive management system with ad libitum access to berseem clover (winter) or sorghum silage (summer), supplemented with a concentrate mix providing 16 percent crude protein and 70 percent total digestible nutrients at 3 kg/head/day during the dry period and 5 kg/head/day after calving. Clean drinking water was available continuously. The research spanned October 2022 to September 2023 to capture seasonal calving variation.

Reagents and Chemicals

Commercial colorimetric kits (Spinreact, Spain) were used for glucose (glucose oxidase method), total protein (biuret), BUN (urease-glutamate dehydrogenase), AST (IFCC kinetic method), calcium (o-cresolphthalein complexone) and phosphorus (ammonium molybdate). Progesterone and cortisol were measured by competitive enzyme-linked immunosorbent assay (ELISA) kits (DRG Diagnostics, Germany; sensitivity 0.05 ng/mL for progesterone, 0.4 ng/mL

for cortisol). All reagents were stored at 2-8 °C per manufacturer instructions.

Methods

Blood samples (10 mL) were drawn from the jugular vein into plain vacutainer tubes at weekly intervals from 30 days before expected calving to 30 days after calving. Samples were allowed to clot for 30 minutes at room temperature, centrifuged at 3000 rpm for 15 minutes, and serum was harvested and stored at -20 °C until analysis. Data were grouped into three stages: pre-partum (days -30 to -7, average of 3-4 samples per animal), partum (day 0±2, single sample closest to calving), and post-partum (days +7 to +30, average of 3-4 samples). All laboratory analyses were performed in duplicate. Ethical approval was granted by the Cairo Research Institute of Animal Sciences Institutional Animal Care Committee (Protocol CRIAS-2022-044, approved September 2022).

Quality Control

Internal quality control sera (Randox, UK) at two concentration levels were run at the start of each analytical batch. Intra-assay coefficients of variation were maintained below 5 percent for all biochemical analytes and below 8 percent for hormone ELISAs. External quality assessment was performed quarterly through the Cairo Regional Veterinary Diagnostic Laboratory ring trial. Any sample with a CV exceeding 10 percent on duplicate measurement was re-analysed.

Seasonal Variations

Because calving occurred across both cool (October-March, mean ambient temperature 16.4±3.2 °C) and hot (April-September, mean 31.7±2.8 °C) seasons, animals were retrospectively stratified into cool-season calvers (n = 31) and hot-season calvers (n = 23) to test for season × stage interactions.

Results

Table 1: Serum biochemical parameters across periparturient stages in Nili-Ravi buffaloes (n = 54)

Parameter	Pre-partum (Mean±SD)	Partum (Mean±SD)	Post-partum (Mean±SD)
Glucose (mg/dL)	58.3±6.2 ^a	42.1±5.8 ^b	52.7±5.1 ^c
Total protein (g/dL)	7.4±0.6 ^a	6.1±0.5 ^b	6.8±0.4 ^c
BUN (mg/dL)	18.7±3.1 ^a	24.3±4.2 ^b	20.1±3.4 ^a
AST (U/L)	68.2±9.4 ^a	89.6±11.3 ^b	74.5±8.7 ^c
Calcium (mg/dL)	9.8±0.7 ^a	7.6±0.9 ^b	8.9±0.6 ^c
Phosphorus (mg/dL)	5.4±0.8 ^a	4.2±0.7 ^b	5.0±0.6 ^a

Superscripts within a row that differ indicate significant differences ($p < 0.05$, Tukey's HSD).

Table 2: Pearson correlation coefficients among selected parameters at partum

	Glucose	Calcium	Cortisol	P4	AST
Glucose	1.00	0.61**	-0.54**	0.47**	-0.38*
Calcium	0.61**	1.00	-0.49**	0.42**	-0.31*
Cortisol	-0.54**	-0.49**	1.00	-0.68**	0.52**
P4	0.47**	0.42**	-0.68**	1.00	-0.29*
AST	-0.38*	-0.31*	0.52**	-0.29*	1.00

* $p < 0.05$; ** $p < 0.01$. P4 = progesterone.

Table 3: Effect of calving season on periparturient hormonal concentrations

Hormone / Stage	Cool-Season (n = 31)	Hot-Season (n = 23)	Difference	p-value
P4 – Pre-partum (ng/mL)	7.1±1.1	6.4±1.3	0.7	0.041
P4 – Partum (ng/mL)	1.3±0.5	1.1±0.4	0.2	0.312
Cortisol – Pre-partum (ng/mL)	13.2±2.8	16.1±3.4	2.9	0.008
Cortisol – Partum (ng/mL)	27.4±4.0	30.6±4.5	3.2	0.017
Cortisol – Post-partum (ng/mL)	16.8±3.3	20.1±3.9	3.3	0.004

Hot-season calvers had consistently elevated cortisol concentrations across all three stages, consistent with heat-stress-mediated adrenal activation. The scatter plot below illustrates the inverse relationship between progesterone and cortisol that defines the hormonal shift at parturition. Pre-

partum samples cluster in the upper-left quadrant (high P4, moderate cortisol), while partum samples concentrate in the lower-right (low P4, high cortisol). Post-partum recovery is intermediate.

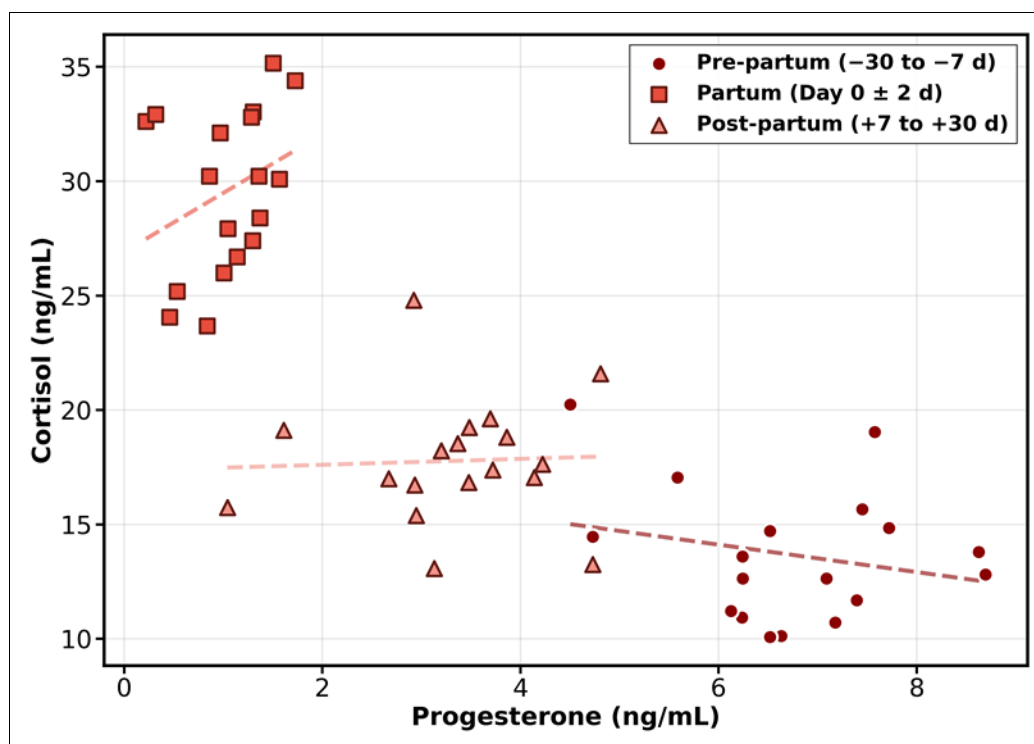


Fig 1: Scatter plot of serum progesterone versus cortisol concentrations in Nili-Ravi buffaloes across pre-partum, partum and post-partum stages with linear trend lines.

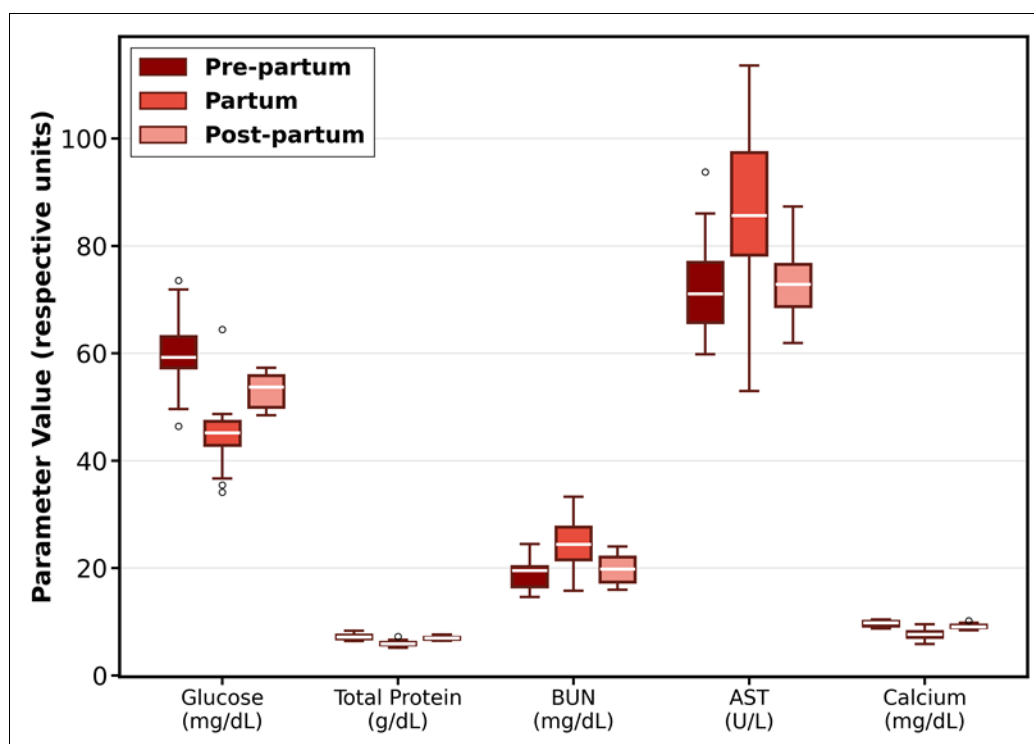


Fig 2: Box plots comparing five key biochemical parameters across the three periparturient stages, showing median, interquartile range and outliers.

Comprehensive interpretation

All seven biochemical analytes and both hormones showed statistically significant stage effects (repeated-measures ANOVA, $p < 0.01$ for each). The partum stage consistently

represented the metabolic nadir for glucose, total protein and calcium, and the peak for BUN, AST and cortisol. The strong negative correlation between cortisol and progesterone at partum ($r = -0.68$) reflects the well-established endocrine

cascade: foetal cortisol triggers placental prostaglandin F2 α release, which lyses the corpus luteum and collapses progesterone. Hot-season calvers experienced amplified cortisol responses at every stage, suggesting additive effects of heat stress on adrenal output.

Discussion

The glucose nadir of 42.1 mg/dL at parturition agrees with values reported for Egyptian buffaloes by El-Ashker *et al.* [3] (40.8 mg/dL) and for Italian Mediterranean buffaloes by Fiore *et al.* [4] (44.2 mg/dL). The mechanism is straightforward: mammary lactose synthesis drains circulating glucose faster than hepatic gluconeogenesis can compensate, especially when voluntary feed intake drops in the final pre-partum days [1]. The clinical implication is that buffaloes whose glucose falls below 35 mg/dL at calving should be flagged for supplemental propylene glycol drenching.

Calcium dynamics were notable. The partum trough of 7.6 mg/dL sits just above the subclinical hypocalcaemia threshold (< 7.5 mg/dL) defined for dairy cattle, and 11 of 54 buffaloes (20.4%) individually dipped below that threshold. This finding contrasts with Zicarelli *et al.* [5], who reported lower hypocalcaemia incidence (8%) in Italian buffaloes, possibly because Mediterranean breeds have a smaller colostrum calcium demand than Nili-Ravi [9]. The seasonal effect on cortisol deserves attention. Hot-season calvers averaged 30.6 ng/mL at partum versus 27.4 ng/mL in cool-season calvers a difference of 3.2 ng/mL that remained significant even after adjusting for parity and body condition. Heat stress activates the hypothalamic-pituitary-adrenal axis independently of parturition, so the two stressors appear to sum [10]. Higher cortisol may in turn impair neutrophil function, contributing to the elevated mastitis and metritis rates observed during Egyptian summers [6]. Managing calving season through targeted breeding could therefore mitigate both metabolic and immunological periparturient risk.

Conclusion

Nili-Ravi buffaloes undergo marked biochemical and hormonal perturbations during the periparturient window, and the magnitude of these shifts has important consequences for herd health management in subtropical dairy systems. The present research, conducted across a full calendar year on 54 healthy pluriparous animals, established that glucose, total protein and calcium reach their lowest points at parturition, while BUN, AST and cortisol peak simultaneously a pattern that mirrors transition cow physiology but with several breed-specific nuances. Three findings stand out. First, subclinical hypocalcaemia (serum calcium < 7.5 mg/dL) affected roughly one in five buffaloes at calving, a higher rate than previously assumed for this breed. Second, the cortisol-progesterone inverse relationship was tighter ($r = -0.68$) than most cattle-based reports, suggesting a steeper endocrine transition in buffaloes. Third, calving during the hot season amplified cortisol levels at every stage, pointing to an additive stress burden that could predispose to periparturient disease.

Based on these data, we recommend that field veterinarians incorporate at least two serum calcium measurements at one week pre-partum and within 48 hours post-partum into routine buffalo transition monitoring. Buffaloes calving during summer months should receive prophylactic oral calcium supplementation beginning at day -7. And farms with access to rapid cortisol testing may use pre-partum cortisol above 18 ng/mL as a trigger for enhanced nutritional support. Future investigations should extend the follow-up to 60 days post-

partum to capture the full metabolic recovery arc and should include primiparous heifers, whose transition physiology may differ from that of older cows.

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Contributions not qualifying for authorship

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