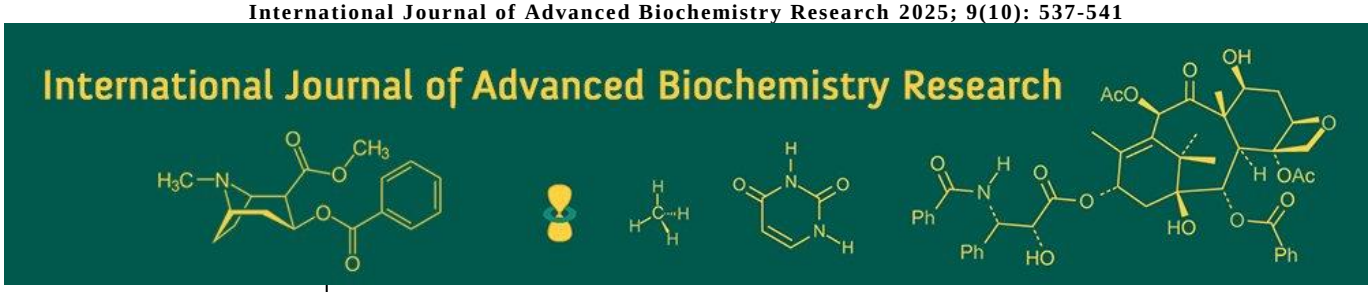

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Hemato biochemical and mineral alterations in cows affected with subclinical ketosis

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Abstract
Subclinical ketosis (SCK) is a prevalent metabolic disorder in high-yielding dairy cattle during the early postpartum period. This study aimed to evaluate the hemato-biochemical and mineral profile changes in crossbred cows affected with SCK during the periparturient period. A total of 101 cows were screened, among which 8 were diagnosed with SCK based on blood β-hydroxybutyrate (BHBA) >1.2 mmol/L and non-esterified fatty acids (NEFA) >0.4 mmol/L. Blood samples were collected during three physiological stages: far-off dry (FOD), close-up dry (CUD), and fresh. Hematological, biochemical, and mineral parameters were analyzed using automated analyzers. Hematological indices such as hemoglobin, PCV, and TEC showed non-significant declines, while leukocytosis was observed at the fresh stage. Significant decreases in total plasma protein and glucose levels were noted in the fresh stage, while NEFA and BHBA levels increased. Plasma mineral concentrations revealed non-significant variations, although trends suggested decreased calcium and phosphorus levels in fresh stage cows. These findings support that SCK is associated with marked metabolic disturbances that can be used for early detection and management.

Keywords: Subclinical ketosis, β-hydroxybutyrate, NEFA, biochemical parameters, minerals

Introduction
Subclinical ketosis (SCK), also known as hyperketonemia, is an important metabolic disorder in high-producing dairy cows, particularly during the early postpartum period. It is characterized by elevated concentrations of circulating ketone bodies—primarily β-hydroxybutyrate (BHBA). (Mc Cart *et al.*, (2012) [1].
Unlike clinical ketosis, subclinical ketosis remains undetected without diagnostic testing, typically relying on blood BHBA levels ≥1.2 mmol/L as the threshold (McArt *et al.*, 2012a) [1]. Due to the absence of clinical signs, animals in SCK continue to produce at a reduced rate resulting in reduced milk yield, impaired reproductive performance, increased risk of displaced abomasum, metritis, mastitis, and elevated culling rates (Duffield *et al.*, 2009; Walsh *et al.*, 2007) [2, 3].
So in order to study the impact of SCK on various metabolic parameters, a study was planned to study the effect on various metabolic parameters viz haematological, biochemical and various mineral parameters.

Materials and Methods
Sample Collection
In every selected farm, crossbred cows which were in their last trimester of pregnancy and was dried off milking were selected for the study. Out of total 101 cows sampled, 08 cows were diagnosed with sub clinical ketosis on the basis of blood BHBA > 1.2 mmol/L and NEFA >0.4 mmol/L during the fresh period.
From each animal blood samples were collected thrice during different stages of periparturient period, viz:

Far off dry: > 10 days following dry off and not < 30 days prior to calving.
Close up dry: Between 3 and 21 days prior to calving.
Fresh: 3 to 30 days in milk.

Laboratory Analysis

Hamatology

Haematological parameters were estimated on the haematological analyser (ADVIA 2120, SIEMENS HematologyAnalyzer, USA.) in the Diagnostic Laboratory of Department of Teaching Veterinary Clinical complex, GADVASU, Ludhiana.

Mineral analysis

Concentrations of various minerals viz. Cu, Zn and Fe were estimated by Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 700, USA). Concentrations of minerals viz. Ca, Mg, Na and K were estimated by Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 700, USA). Plasma Pi was determined using method given by Tausky and Shorr, (1953) [4].

BHBA AND NEFA

Both BHBA and NEFA were estimatedon the ELISA plates from the plasma samples with the help of kits provided by DiasysDiagnostics systems, Germany.

Biochemistry

Estimation of Biochemical parameters viz.Total Plasma Proteins (TPP), albumin, plasma urea nitrogen (PUN), creatinine, glucose were analysed by kits provided by Ortho clinical diagnostics, UK. on the semi automatic Biochemistry analyzer (Vitros®DT 60 II by Ortho clinical diagnostics USA.) in the Diagnostic Laboratory of Department of Teaching Veterinary Clinical complex.

Statistical Analysis

Data was analysed by one way analysis of variance (ANOVA) andpaired t-test for comparison. The Linear regression for calculating the prediction equation and correlation between different groups were obtained using SPSS software (version 16.0; Microsoft).

Results

The mean hematological indices of cows and buffaloes suffering from SCK are presented in Table 1 respectively. The mean Hb value of cows suffering from SCK shows a non significant decline from FOD to fresh stage. The mean PCV value show no significant change in PCV of SCK affected cows.

Total Erythrocyte count and Total Leucocyte Count

The mean TEC count does not show any significant change, whereas, marked leukocytosis was observed at fresh stage in SCK affected cows.

Table 1: Hematological indices in crossbred cows suffering from SCK

Parameters	Period	FOD	CUD	Fresh
Hb (g/dl)		10.45 ± 0.50^{A}	9.90 ± 0.38^{A}	9.40 ± 0.39^{A}
PCV (%)	FOD	30.70 ± 1.82^{A}	29.75 ± 1.78^{A}	27.35 ± 1.72^{A}
TEC ($x10^{6}/\mu l$)	FOD	5.91 ± 0.22^{A}	5.63 ± 0.27^{A}	5.17 ± 0.32^{A}
TLC ($x10^{3}/\mu l$)	FOD	9.31 ± 0.56^{A}	9.92 ± 0.35^{A}	11.44 ± 0.18^{B}
Values bearing different superscript in capital alphabets (A, B, C) across the rows differ significantly ($p<0.05$)				

Biochemical Parameters

The mean biochemical parameters of cows suffering from SCK are provided in Table 2.

Mean TPP level declined significantly from FOD/CUD to fresh stage.

Plasma urea Nitrogen (PUN)

No significant change was observed in mean PUN levels in SCK affected cows.

Creatinine

No significant change was observed in SCK affected cows.

Glucose

Significant decline was observed from FOD and CUD to fresh stage in SCK affected cows.

Non Esterified Fatty Acids (NEFA)

Significant increase was observed from FOD to CUD and fresh stage in SCK affected cows. Levels of NEFA were significantly high throughout transition in SCK affected cows.

Table 2: Biochemical profile in crossbred cows suffering from SCK (Mean ± S.E.)

Parameters	FOD	CUD	Fresh
TPP (g/dl)	6.42 ± 0.14^{A}	6.11 ± 0.22^{AB}	5.72 ± 0.20^{B}
AAlbumin (g/dl)	2.65 ± 0.12^{A}	2.50 ± 0.17^{A}	2.22 ± 0.20^{A}
PUN (mg/dl)	12.57 ± 2.08^{A}	13.14 ± 1.98^{A}	14.65 ± 1.76^{A}
Creatinine (mg/dl)	1.40 ± 0.13^{A}	1.61 ± 0.15^{A}	1.54 ± 0.17^{A}
Glucose (mg/dl)	65.71 ± 3.79^{A}	58.14 ± 4.74^{A}	44.00 ± 2.98^{B}
BHBA (mmol/L)	0.41 ± 0.08^{A}	0.63 ± 0.05^{A}	1.35 ± 0.12^{B}
NEFA (mmol/L)	0.41 ± 0.04^{A}	0.81 ± 0.08^{B}	1.01 ± 0.09^{B}
Values bearing different superscript in capital alphabets (A, B, C) across the rows differ significantly ($p<0.05$)			

Concentration of Plasma Minerals in Cows suffering from Sub Clinical Ketosis (SCK)

The mean plasma mineral profile in SCK affected cows are provided in Table 3

Calcium (Ca)

The mean calcium levels in SCK affected cows show non significant decrease from FOD to fresh period.

Magnesium (Mg)

No significant change was observed in SCK affected cows

Sodium (Na)

No significant change was observed in Na levels SCK affected cows.

Potassium (K) in cows

No significant change was observed in K levels in SCK affected cows. Levels of K were significantly low in SCK affected buffaloes during FOD and CUD stage as compared to healthy controls but were within normal range. It is imperative that some degree of hypokalemia will occur in most dairy animals as they generally suffer a reduction in feed intake in early lactation, and they begin to produce milk rich in potassium and in case of SCK the resultant reduced feed intake is more severe.

Copper (Cu)

Non significant decrease was observed in cu levels from FOD to fresh period in cows affected with sub clinical ketosis.

But, Zhang *et al.*, (2010) [5] did not observe any effect of subclinical ketosis on the mean plasma Cu levels in the healthy and SCK affected cows

Iron (Fe)
Non significant decrease was observed in Fe levels from FOD to fresh period in cows affected with sub clinical ketosis.

Zinc (Zn)
No significant change was observed throughout transition.

Inorganic Phosphorus (Pi) in cows
No significant change was observed throughout transition.

Table 3: Plasma minerals concentration in cross breed cows suffering from SCK

Parameters	FOD	CUD	Fresh
Ca (mmol/l)	2.03 ± 0.14^{A}	2.01 ± 0.13^{A}	1.96 ± 0.13^{A}
Mg (mmol/l)	1.10 ± 0.10^{A}	1.09 ± 0.11^{A}	1.05 ± 0.11^{A}
Na (mmol/l)	141.43 ± 2.42^{A}	141.14 ± 2.56^{A}	140.29 ± 1.93^{A}
K (mmol/l)	4.64 ± 0.19^{A}	4.55 ± 0.18^{A}	4.47 ± 0.16^{A}
Cu (µmol/)	10.13 ± 0.74^{A}	9.98 ± 0.76^{A}	9.38 ± 0.86^{A}
Fe (µmol/)	117.12 ± 4.08^{A}	116.10 ± 4.17^{A}	114.87 ± 5.32^{A}
Zn (µmol/)	16.12 ± 0.93^{A}	15.74 ± 0.87^{A}	15.18 ± 0.81^{A}
Pi (mmol/l)	1.55 ± 0.05^{A}	1.53 ± 0.05^{A}	1.52 ± 0.05^{A}
Values bearing different superscript in capital alphabets (A, B, C) across the rows differ significantly ($p<0.05$)			

Discussion

Hematological Indices

Hemoglobin (Hb)
Although statistically insignificant, this reduction may be linked to hemodilution commonly observed during the periparturient period. Decreased Hb can impair oxygen delivery to tissues, potentially contributing to fatigue and metabolic inefficiency in affected cows.

Packed Cell Volume (PCV)
The PCV values followed a similar trend, with a slight decline from FOD to fresh stage. This trend indicates mild anemia or hemodilution, often observed during early lactation due to plasma volume expansion.

Total Erythrocyte Count (TEC)
TEC also declined progressively through the transition period, though not significantly. This decline may be attributed to the physiological adjustments during parturition and early lactation, reflecting reduced erythropoiesis or mild anemia associated with metabolic disorders.

Total Leukocyte Count (TLC)
Marked leukocytosis was observed at the fresh stage ($11.44\pm0.18 \times 10^3/\mu l$), suggesting a stress or inflammatory response. The elevated TLC aligns with reports that metabolic imbalances such as SCK may induce systemic inflammation or immune activation.
These hematological changes were in agreement with Sahinduran *et al.*, (2010) [6], who observed no significant hematological deviations in ketotic cows, indicating that SCK may not always result in overt hematologic abnormalities but could contribute to subtle systemic changes.

Biochemical Profile

Total Plasma Protein (TPP) and Albumin
The reduction in TPP and albumin could be due to hepatic lipidosis and impaired protein synthesis during SCK. Decreased plasma proteins often indicate a compromised liver function, a common consequence of excessive fat mobilization in ketotic cows. Similar findings were reported by Djokovic *et al.*, (2013) [7] and Bobe *et al.*, (2004) [8]. Similarly Gonzales *et al.*, (2011) [9], Singh *et al.*, (2019) [10] also observed a decrease in plasma albumin, total proteins and urea in cows with subclinical ketosis.

Plasma Urea Nitrogen (PUN)
No significant change in PUN levels was observed across stages. Greater urea concentration in SCK could be a result of muscle protein catabolism when large amounts of body reserves get mobilized for meeting the negative energy demand. Similarly, Shwartz *et al.*, (2009) [11] and Fekry *et al.*, (1989) [12], Singh *et al.*, (2016) [13] reported that stressed cows had increased PUN levels as compared to control, which could be due to the higher utilization of amino acids as energy source. Also in late gestation, glucose availability for oxidation is supplemented by increased catabolism of amino acids at the expense of protein synthesis, thus increasing urea production.

Creatinine
Creatinine levels remained relatively stable throughout the study period
Glucose
Hypoglycemia in early lactation is a key predisposing factor for SCK. Low glucose levels trigger fat mobilization from adipose tissues, which results in increased NEFA and BHBA production. The mean BHBA level in SCK affected cows after parturation crossed 1.2 mmol/L mark which is the upper limit for defining healthy animals and is standard for diagnosing SCK. A number of studies (McArt *et al.*, 2012a: McArt *et al.*,2012b: Ribeiro *et al.*, 2013) [1, 14, 15] have also reported results similar to the present findings where they classified a cow affected with SCK on the basis of blood BHBA level above 1.2 mmol/L.

BHBA and NEFA
BHBA and NEFA concentrations increased significantly in the fresh stage, reaching 1.35 ± 0.12 mmol/L and 1.01 ± 0.09 mmol/L, respectively. These elevated values clearly confirm the diagnosis of SCK. Increased NEFA levels reflect excessive lipid mobilization, while elevated BHBA levels signify an overwhelmed hepatic capacity for complete oxidation of fatty acids. The findings are consistent with McArt *et al.*, (2012b) [14], who suggested BHBA $\geq$1.2 mmol/L as a threshold for subclinical ketosis.
Together, these findings strongly support the diagnosis of SCK and indicate that major alterations occur in energy metabolism, even in the absence of clinical symptoms. Biochemical markers like glucose, BHBA, NEFA, and TPP are therefore valuable diagnostic indicators of SCK.
This increase in NEFA and BHBA in cows and buffaloes with SCK was ascribed to increased fat mobilization from the adipose tissue during the early lactation period to support the negative energy balance, when blood glucose levels were low due to the initiation of milk production, resulting in increased production of acetyl-CoA, which resulted in increased production of ketone bodies (Wieland *et al.*, 1964) [16].

Plasma Mineral Profile

Calcium (Ca)

Calcium levels showed a non-significant decrease during the transition from FOD (2.03±0.14 mmol/L) to fresh (1.96±0.13 mmol/L). Hypocalcemia may impair smooth muscle function and reduce feed intake, indirectly contributing to SCK. Furthermore, subclinical hypocalcemia can predispose cows to other periparturient disorders, compounding the impact of SCK.

Reduction in the concentrations of Ca and P observed in SCK cows could be due to decreased Ca uptake which might affect the appetite and decrease its absorption from the intestine (Moore, 1997) [17]. Additionally, Ca level can also be reduced due to increased loss of base in the urine to compensate for the acidosis reported in cows with ketosis (Radostits *et al.*, 2000) [18]. More over this decrease in Ca levels in buffaloes coincides with subsequent reduced levels of Mg and it is a well known fact that hypomagnesaemia affects Ca metabolism in two ways i.e., by reducing PTH secretion in response to hypocalcaemia and by reducing tissue sensitivity to PTH. PTH secretion is normally increased greatly in response to even slight decrease in blood Ca concentration but hypomagnesaemia can blunt this response (Littledike *et al.*, 1983) [19].

Magnesium (Mg), Sodium (Na), and Potassium (K)

These minerals did not show significant alterations. However, the downward trend in potassium may indicate reduced intake or losses via milk secretion. Hypokalemia during early lactation is common and can exacerbate the effects of ketosis. Mg is essential for calcium homeostasis, and although not significantly altered, even minor fluctuations can affect parathyroid hormone action.

Copper (Cu), Iron (Fe), Zinc (Zn), and Inorganic Phosphorus (Pi)

Levels of trace elements declined mildly but were statistically insignificant. The role of these minerals in antioxidant defense and energy metabolism is well documented. Deficiencies, even if marginal, may impair liver function and immune response, exacerbating metabolic disorders. Zhang *et al.* (2010) [5] also observed decreased Zn in SCK cows, although our findings did not reflect significant shifts.

Conclusion

The present study highlights that subclinical ketosis in crossbred cows during the periparturient period is associated with significant biochemical disruptions and moderate alterations in hematological and mineral profiles. While hematological changes were mild, the biochemical profile clearly indicated energy deficit and metabolic distress, which were further supported by elevated BHBA and NEFA levels. Mineral fluctuations, though mostly non-significant, may play a supportive role in the pathogenesis and should be considered in preventive nutritional strategies. Early monitoring of glucose, NEFA, BHBA, and plasma proteins can help in timely diagnosis and management of subclinical ketosis.

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