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Haematological and biochemical changes in subclinical ketosis affected cross bred cows in and around Bangalore

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Abstract:
Recently calved cows (0-2 months) were subjected to blood BHBA estimation and cases with blood BHBA level between the ranges of 1400µmol/L to 2500µmol/L were selected as positive for subclinical ketosis. Cows which were apparently healthy and blood BHBA level less than 1000µmol/L were selected as negative for subclinical ketosis and these animals were selected as control group. Blood samples were collected and subjected for estimation of various haematological and biochemical parameters. No significant difference was noticed in haematological parameters like Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), and thrombocyte counts except significant decrease in Haemoglobin (Hb) in subclinical affected letotic cows. Significant decrease in serum glucose, calcium, total protein and albumin with increased AST and GGT is observed in subclinical ketotic cows compare to normal healthy cows.

Keywords: Haematology, biochemical changes, subclinical ketosis

Introduction
Subclinical ketosis may be defined as a preclinical stage of ketosis characterized by an elevated ketone body level without clinical signs such as loss of appetite, hard feces, or dullness. Consequently, it can be confirmed only by qualitative or quantitative analysis of body fluids (Anderson, 1988) [2]. The disease runs sub-clinically; therefore it might be called as silent profit robber on account of its impact on the profitability of dairy farm by milk production loss (around 300kg per lactation), reproduction disturbances (low conception rate, increased artificial insemination), and high risk for developing abomasum displacement, metritis, mastitis and clinical ketosis. Subclinical ketosis can be detected by analyzing blood glucose, blood nonesterified fatty acids (NEFA) and ketone bodies in blood, milk and urine (Anderson, 1988) [2].
The gold standard diagnostic test for subclinical ketosis is the measurement of blood BHBA levels in serum or plasma. By the cowside BHBA test using a hand held meter confers higher levels of sensitivity and specificity than other cowside tests and can replace the need for submitting blood samples to laboratories for BHBA testing (Zhang *et al.*, 2011) [16].
Constable *et al.* (2017) [3] reported that the BHBA is the predominant circulating ketone body. The normal cow have plasma BHBA concentrations less than 1000µmol/L, cows with subclinical ketosis have concentrations greater than 1400µmol/L and cows with clinical ketosis have concentrations often in excess of 2500µmol/L.
Published work on various aspect of subclinical ketosis in cows of Karnataka are limited no concerted efforts have been directed to correlate the blood biochemical parameters with blood ketones. Hence, the present research was taken to estimate and correlate some of the apparently changing biochemical parameters with the blood ketone levels and to find out a suitable marker which subsequently can be used as a diagnostic test for impending subclinical ketosis in lactating dairy cows.

Material and Methods
Recently calved cows (0-2 months) belonging to the Veterinary College dairy farm Bengaluru, outdoor patients brought for treatment at Veterinary College Hospital, Bengaluru and

individual animals shown by owners at their holdings in and around Bengaluru were examined for subclinical ketosis during the year October and November 2019.These cows were subjected to blood BHBA estimation and cases with blood BHBA level between the ranges of 1400µmol/L to 2500µmol/L were selected as positive for subclinical ketosis. Cows within two months of calving which were apparently healthy and blood BHBA level less than 1000µmol/L were selected as negative for subclinical ketosis and these animals were selected as control group.

Group I: (Control group): Recently calved apparently healthy cows, showing blood BHBA level lower than 1000µmol/L.

Group II: Animals found positive for subclinical ketosis. Cases with blood BHBA level between the range of 1400µmol/L to 2500µmol/L were selected as positive for subclinical ketosis.

Blood BHBA: Blood BHBA was estimated by using Freestyle Optium Neo H Blood glucose and ketone monitoring system (Abbott Laboratories, UK) and Freestyle Optium H blood beta ketone test strips (Abbott Laboratories, UK) as described by Schade DS and Eaton RP (1982) [14]. A small drop of blood from ear vein was instilled on a disposable ketone test strip of the meter and after few seconds results were recorded and expressed in mmol/L and convert it toµmol/L by multiplying the results with 10^{-3}.
Detailed clinical examination was carried out for each animal as per the standard methods suggested by Kelly (1984) [7]. The rectal temperature of all the animals was recorded in ºF. The pulse rate was recorded over the middle coccygeal artery and expressed as rate per minute, respiration was counted by observing the nostril movements and heart rate was recorded in beats per minute. The rumeno-reticular motility was recorded by directly placing the fist on left flank and rumen motility counted for three minutes. The milk yield per day of these animals were also recorded.

Haematology: Haemoglobin (Hb), Total Erythrocyte Count (TEC), Total Leukocyte Count (TLC), and thrombocyte counts were estimated using Auto analyzer/Cell counter by Mindray (BC-2800vet).

Serum biochemistry: Blood was collected from healthy and affected groups from jugular vein using serum vacutainers under aseptic conditions. After collection, blood was allowed to clot at room temperature and centrifuged for serum separation. Sera samples were transferred into Eppendorf tubes and were maintained at 4 ºC and all the biochemical findings were estimated on same day of collection. The following biochemical parameters were estimated using Semi-automatic biochemistry analyzer RX-50 (Microlab) and reagents manufactured by Transasia Bio-Medicals Ltd, Solan, HP. Viz., Glucose, Calcium, Total protein, Albumin, Aspartate aminotransferase (AST), Gamma GlutamylTransferase (GGT)

Statistical analysis: Statistical analysis was performed using the statistic software Graphpad Prism version 7.0 for windows. Mean values and standard error were calculated and all values were expressed as mean ±SE. The data were analyzed by analysis of variance (ANOVA).

Results

Blood beta hydroxy butyric acid (BHBA): In the present study, the mean ±SE of BHBA in Group I was 633.33±55.78µmol/L. The mean ± SE of BHBA in Group II was 1766.67±76.01µmol/L. There was a significant increase in BHBA values in sub-clinical ketosis affected cows.

Haemoglobin: In the present study, the mean ± SE of haemoglobin in Group I was 10.52±0.2g/dl. The mean ± SE of haemoglobin in Group II was 8.17±0.11g/dl, There was a significant (P≤0.05) decrease in haemoglobin in sub-clinical ketosis affected cows.

Total Erythrocyte Count (TEC): In the present study mean ±SE of TEC in Group I was $6.67\pm0.22\times10^{6}$/µL. The mean ±SE of TEC in Group II was $6.48\pm0.21\times10^{6}$/µL. There was no significant difference observed between the two Groups.

Total Leukocyte Count (TLC): In the present study mean ±SE of TLC in Group I was $9.12\pm0.52\times10^{3}$/µL. The mean ±SE of TLC in Group II on was $10.1\pm0.45\times10^{3}$/µL, there was no significant difference observed between the two groups

Thrombocyte count: In the present study mean ±SE of thrombocytes in Group I was $400.83\pm28.33\times10^{3}$/µL. The mean ±SE of thrombocytes in Group II was $382.17\pm16.14\times10^{3}$/µL. There was no significant difference observed between the two groups

Serum glucose: In the present study, the mean ± SE of serum glucose in Group I was 55.28±0.64mg/dL. The mean ± SE of serum glucose in Group II was 44.8±1.58mg/dL. There was a significant decrease in serum glucose in Group II when compared to Group I.

Serum calcium: In the present study, the mean ± SE of serum calcium in Group I was 10.35±0.12mg/dL. The mean ± SE of serum calcium in Group II was 9.47±0.17mg/dL. There was a significant (P≤0.05) decrease in serum calcium in Group II when compared to Group I.

Aspartate Aminotransferase (AST): In the present study, the mean ± SE of serum AST in Group I was 87.65±3.19U/L. The mean ± SE of serum AST in Group II was 116.58±7.82U/L. There was a significant (P≤0.05) increase in serum AST in Group II when compared to Group I.

Gama-glutamyltransferase (GGT): In the present study, the mean ± SE of serum GGT in Group I was 15±0.49U/L. The mean ± SE of serum GGT in Group II was 31.27±1.57U/L. There was a significant (P≤0.05) increase in serum GGT in Group II when compared to Group I.

Serum total protein: In the present study, the mean ± SE of serum total protein in Group I was 7.35±0.12g/dL. The mean ± SE of serum total protein in Group II was 5.85±0.21g/dL. There was a significant (P≤0.05) decrease in serum total protein in Group II when compared to group I.

Albumin: In the present study, the mean ±SE of serum albumin in Group I was 2.27±0.05g/dL. The mean ±SE of serum albumin in Group II was 1.88±0.05g/dL. There was significant decrease in serum albumin in group II when compared to group I.

Table 1: Comparision of BHBA and various Hematological and Biochemical parameters between healthy and subclinical ketosis affected cows

Parameters	Group I(Healthy)	Group II(Sub-clinical ketosis)
Beta hydroxybutyric acid (BHBA) (µmol/L)	633.33±55.78^{ax}	1766.67±76.01^{bx}
Haemoglobin (gm/dL)	10.52±0.2^{ax}	8.17±0.11^{bx}
Total erythrocyte count (×10^6/µL)	6.67±0.22^{ax}	6.48±0.21^{ax}
Total leucocyte count (×10^3/µL)	9.12±0.52^{ax}	10.1±0.45^{ax}
Thrombocytes (×10^3/µL)	400.83±28.33^{ax}	382.17±16.14^{ax}
Serum glucose (mg/dL)	55.28±0.64^{ax}	44.8±1.58^{bx}
Serum calcium (mg/dL)	10.35±0.12^{ax}	9.47±0.17^{bx}
Aspartate Aminotransferase (AST) (U/L)	87.65±3.19^{ax}	116.58±7.82^{bx}
Gamma-glutamyltransferase (GGT) (U/L)	15±0.49^{ax}	31.27±1.57^{bx}
Serum total protein (g/dL)	7.35±0.12^{ax}	5.85±0.21^{bw}
Serum albumin (g/dL)	2.27±0.05^{axy}	1.88±0.05^{bx}

$^{a, b,}$ Mean values in a row with different superscripts differ significantly (P≤0.05)

$^{x, y,}$ Mean values in a column with different superscripts differ significantly (P≤0.05)

Discussion

Blood beta hydroxy butyric acid: There was a significant increase in blood BHBA in animals suffering with SCK animals when compared with healthy animals. Similar findings reported by Rodriguez-Jimenez *et al.* 2018 and Djokovic *et al.*, 2019) [10, 4]. This could be due a dramatic increase in energy requirements during the late pregnancy and early lactation making dairy cows highly susceptible to negative energy balance. A majority of cows cannot meet their energy requirements for milk production and are forced to mobilize body fat to meet their energy needs. When large amount of body fat are utilised as an energy source to support production, fat is sometimes mobilized faster than the liver can properly metabolise it. If this situation occurs, ketone production exceeds ketone utilisation by the cow and ketosis results when gluconeogenic precursors are limiting. Ketone bodies provide energy to peripheral tissues when carbohydrates are limiting. The circulating ketone bodies are acetoacetate (AcAc), betahydroxybutyrate (BHB) and acetone (Ac), where acetoacetate (AcAc) is the parent ketone body, which can be reduced to beta-hydroxybutyrate (BHB) in an enzymatic reaction or decarboxylated to acetone (Ac) in a spontaneous non-enzymatic reaction. In subclinical ketosis affected cows, BHBA is the predominant circulating ketone body and is relatively stable in whole body, plasma or serum.

Haematological parameters

Haemoglobin (g/dL): There is a significant decrease in haemoglobin in animals suffering with SCK animals when compared with healthy animals. Similar findings reported by Sahoo *et al*. (2009) [12] and Har (2015) [5].

Total Erythrocyte Count (TEC) (×10^6/µl): In the present study, values of total erythrocyte count in subclinical ketosis were within their physiological limits as compared to healthy control group. There was no significant difference observed between the groups. The results of the present study agree with findings of Sahoo *et al*. (2009) [12] and Har (2015) [5] who also observed that there was no significant difference in the total erythrocyte count in animals suffering with subclinical ketosis and healthy animals.

Total Leukocyte Count (TLC) (×10^3/µl): In the present study, the values of total leucocyte count in subclinical ketosis Group II were within their physiological limits as compared to healthy control group. There was no significant difference was observed between the groups. The results of the present study agree with findings of Sahoo *et al*. (2009) [12] and Sahinduran *et al.* (2010) [11] who also observed that there was no significant difference in the total leucocyte count in animals suffering with subclinical ketosis and healthy animals.

Thrombocytes (×10^3/µl): In the present study, the values of thrombocyte count in subclinical ketosis were within their physiological limits as compared to healthy control group. There was no significant difference observed between the groups. The results of the present study agree with findings of Sahoo *et al*. (2009) [12], Sahinduran *et al.* (2010) [11] and Har (2015) [5] who also observed that there was no significant difference in the thrombocyte count in animals suffering with subclinical ketosis and healthy animals.

Serum biochemistry

Serum glucose (mg/dL): In the present study there was a significant decrease in serum glucose in all the animals suffering with SCK compared to healthy animals. Similar reports were given by Kachhawaha *et al.* (2016) [6] and Marutsova *et al.* (2018) [8]. This decrease in serum glucose value is due to many high yielding dairy animals have a negative energy balance during first few weeks of lactation. The demand of mammary gland for glucose is often greater than glucose available. Even though milk flow can be reduced by reduction of energy intake, this does not follow automatically in early lactation, because the hormonal stimuli for mammary activity overcome the effect of reduced feed intake. This imbalance may lead to hypoglycemia with a negative carbohydrate imbalance.

Serum calcium: In the present study there was a significant decrease in serum calcium in all the animals suffering with SCK compared to healthy animals. The results of the present study agree with findings of Padmaja and Rao (2013) [9] and Akgul *et al.* (2017) [1]. The decrease in serum calcium could be due to loss of base in urine to compensate acidosis or due to disturbance in absorption of minerals from gut as per Padmaja and Rao (2013) [9]. Also high concentration of BHBA impairs the absorption and utilization of calcium in dairy cows with subclinical ketosis during the early lactation period and serum calcium concentration can be used as a reference index for the diagnosis of subclinical ketosis. This decline in plasma calcium levels in the late gestation and in the early lactation period is due to the increased transfer of calcium for the development of foetal skeleton and for the milk synthesis during the early lactation period, which along with decreased dry matter intake is not balanced by increase in rate of absorption from gut or mobilization from bone (Singh *et al.*, 2017) [15].

Aspartate Aminotransferase (AST) and Gama-glutamyltransferase (GGT): In the present study, there was a significant increase in the serum AST and GGT in all the groups with SCK compared to healthy animals. The results of the present study agree with the findings of Padmaja and Rao (2013) [12], and Har (2015) [5].The high serum AST and GGT level in post-parturient lactating cattle might be due to excess metabolism of fat and ultimately deposition of fat globules in the hepatocyte and leakage of enzyme in the blood circulation and fat accumulation in the liver resulting in high hepatocytes membrane permeability and is a good tool for early detection of metabolic liver diseases.

Serum total protein and Albumin: In the present study there was a significant decrease in serum total protein in all the groups with SCK compared to healthy animals. The results of the present study agree with findings of Sainath (2015) [13]. Decrease in protein levels in subclinical ketotic animals may be attributed to protein catabolism as the animals suffer from the energy deficit, for an increased rate of gluconeogenesis which serves as an important source of energy for synthesis of milk lactose and milk protein. This decrease in mean plasma glucose and total proteins and albumin levels during early lactation period might be due to low energy diet and reduce dry matter intake during the early lactation period.

Conclusion

Early detection of subclinical ketosis in dairy animals is mandatory to prevent economic loss to the farmers and it can be effectively undertaken by blood BHBA estimation in postpartum cows. The blood BHBA with a cut-off between 1400µol/L to 2400µol/L can be potentially useful tool for the routine monitoring of subclinical ketosis in early postpartum dairy cows. The blood BHBA estimation at field level can be done using hand held meter used as the primary monitoring tool and it can replace the need for submitting blood samples to laboratories for BHBA testing.

The changes in haematology have a limited diagnostic value in subclinical ketosis. Significant decrease in serum glucose, calcium, total protein and albumin with increased AST and GGT is observed in subclinical ketotic cows compare to normal healthy cows.

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