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Detection of sub-clinical ketosis by hand held ketometer

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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. 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.

Keywords: Sub clinical ketosis, hand held ketometer

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) [12].
Constable *et al.* (2017) [4] 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.
The present research was taken to estimate 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 considered as positive for subclinical ketosis. Cows within two months of calving which were apparently healthy and blood BHBA level less than 1000µmol/L were considere as negative 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) [10].

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) [6]. 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.

Plate 1: Freestyle Optium Neo Blood Glucose and Ketones monitoring system and Blood β-ketone test strips

Plate 2: Estimation of blood BHBA value by collecting a drop of blood from ear vein of subclinical ketotic cow

Results and Discussion

Table 1: Mean ± SE values of blood beta hydroxyl butyric acid in different groups

Beta hydroxybutyric acid (BHBA) (µmol/L)	
Groups	**0th day**
Group-I Control	633.33±55.78^{ax}
Group-II Sub-clinical ketosis cows	1766.67±76.01^{bx}

a, b, c, d Mean values in a row with different superscripts differ significantly ($P \leq 0.05$)

w, x, y, z Mean values in a column with different superscripts differ significantly ($P \leq 0.05$)

Note: Sub-clinical ketotic cows have significantly increased Beta hydroxybutyric acid when compared to healthy cows

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 Nazifi *et al.* (2008) [8], Akgul *et al.* (2017) [1], Marutsova *et al.* (2018) [7] Rodriguez-Jimenez *et al.* (2018) [9] and (Djokovic *et al.*, 2019) [5]. 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 (Bergman, 1971 and Youssef *et al.*, 2010) [3, 11]. In subclinical ketosis affected cows, BHBA is the predominant circulating ketone body and is relatively stable in whole body, plasma or serum.

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.

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