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Prevalence of subclinical ketosis in anestrum dairy cows

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Abstract
Subclinical Ketosis, questionably the most significant metabolic disease in dairy cattle due to negative energy balance (NEB) during lactation. NEB reflects directly on the production and metabolic diseases. Estimation of ketones and Non-esterified fatty acids (NEFA) for sub-clinical ketosis in Post-partum anestrum animals reflects the metabolic status of the animal. Follicular dynamics often gets impaired in the cows with ketosis. Ten healthy and ten post-partum anestrum cows are selected for this study. Blood, serum and urine samples were collected and subjected for analysis of NEFA, blood ketones and urinalysis. No significant differences could be observed for NEFA in anestrum animals. However, 80 per cent of anestrum animals showed elevated blood ketones and marked difference in urinary ketones. Hence subclinical ketosis plays a major influence in resumption of postpartum cyclicity and diagnosis of subclinical ketosis by estimation of blood ketones is more reliable than the estimation of NEFA. By using bovine specific ketone-meter the diagnosis of subclinical ketosis especially in the postpartum period is more sensitive and will help in prevention of prolonged anestrum.

Keywords: cattle, ketones, NEFA, post-partum anestrum, subclinical ketosis

Introduction
Metabolic diseases are often subclinical and cause an economic loss for dairy farmers [1]. Negative energy balance is the difference between the energy intake and energy required for maintenance and production [2]. The difference reflects directly on the production and metabolic diseases [3]. However, it is a crucial event immediately after post-partum and if it is unnoticed it may reflect in the resumption of ovarian cyclicity. Estimation of the true energy status of the animal is quite difficult since it requires determination of energy input and determination of energy output in different forms [2]. An appropriate alternative is to measure the metabolites which are associated with the energy status such as non-esterified fatty acids (NEFA) and ketone bodies. The diagnostic techniques have to be reliable and simple enough at the filed level. The study was designed to estimate the ketones and NEFA for sub-clinical ketosis in Post-partum anestrum animals and also to compare with the urinal parameters to find it reliable.

Materials and Methods
Cows addressing to Veterinary College and Research institute, Orathanadu from August 2019 to January 2020, with the history of normal postpartum first estrum animals were selected under Group 1 as control (n=10). The animals that are not coming to estrum 90 days post-partum and free from uterine infection, gross genital tract abnormalities and having good body condition are selected under Group 2 category (n=10). Body condition score (BCS) was estimated as per standards with scores 0-5. Serum samples were collected and subjected for estimation of NEFA by Auto-analyser using specific kit (Randox Laboratories UK) (Fig. 1 &2). Blood ketones were estimated using bovine specific keto check instrument (BHBCheck Plus-Portacheck, Moorestown USA) by obtaining blood sample from the ear vein (Fig. 3 &4). Urinal parameters such as blood, bilirubin, urobilinogen, ketones, proteins, pH, glucose and leucocytes were estimated quantitatively using a urinalyser.

Results and Discussions
The mean (± Standard error) values for estimation of NEFA and blood ketones - blood ß-hydroxy butyric acid (BHBA) are depicted in Table 1. The mean (± Standard error) values for the urinal parameters such as blood, bilirubin, urobilinogen, ketones, proteins, pH, glucose and leucocytes are shown in Table 2.

Table 1: Mean (± Standard Error) for NEFA and BHB for Healthy and Anestrum cows

S No	Group	NEFA (mmol/L)	Blood Ketones (mmol/L)
1	Control	1.2±0.189[a]	1.1±0.124 [a]
2	Anestrum	2.7±0.401[b]	1.6±0.132 [b]

**values within the column with different superscripts differ significantly $P<0.01$

Table 2: Mean (± Standard Error) for Urinalysis in Control and Anestrum Cows

S No	Group n=10	Blood	Bilirubin (mg/100ml)	Urobillinogen (mg/100ml)	Ketone (mg/100ml)	Protein (mg/100ml)	Nitrate	pH	Leucocytes
1	Control	0±0	0.5±0.02	1±0	8.2±1.02 [a]	27.1±1.33	0±0	8.8±0.13	1±0.33
2	Anestrum	0±0	0.3 ±0.03	1±0	11.9±0.92 [b]	25.4±3.00	0±0	8.8±0.13	1±0.39

**values within the column with different superscripts differ significantly $P<0.01$

Even though NEFA and Blood ß-Hydroxy Butyric acid (BHBA) were analyzed in control and Anestrum animals; There is no significant changes could be observed between control and anestrum cows. But 80% of cows in anestrum are showing >1.2 mmol/l of blood ketones. There are no major significant changes could be noticed in urinalysis; however urinary ketones in anestrum cows are signaling for sub-clinical ketosis. Hence BHBA using bovine specific ketonemeter can be an easy tool for assessing sub-clinical ketosis, which could be one of the factors causing postpartum anestrum

Negative energy balance has serious effect on fat metabolism; decreased blood glucose promotes lipolysis in turn release of Non-Esterified fatty acids. In the liver, NEFA may be completely utilized in TCA cycle for ATP or partially utilized via beta oxidation and in turn form ketone bodies such as acetone, acetoacetic acid, BHBA; converted into triglycerides or low density lipoproteins and come out of the liver [4]. In the normal transition period, NEFA and ketone bodies will appear in circulation as an end product of energy source. However, if the level of ketone bodies increases it is harmful to productivity [5]

NEFA is a precursor to ketone bodies and does not appear as threshold level all the time and hence prediction of NEFA may not be a truce indicator in subclinical ketosis; Though determination of ketone bodies among with BHBA will reflect the true status of negative energy. The negative energy status will reflect on delay in the resumption of ovarian cyclicity in all stages of follicular dynamics such as recruitment, selection, Dominance and maturation [6].

Fig 1: Kit for NEFA

Fig 2: NEFA estimation using auto analyzer

Fig 3: BHBcheck Plus ketonemeter

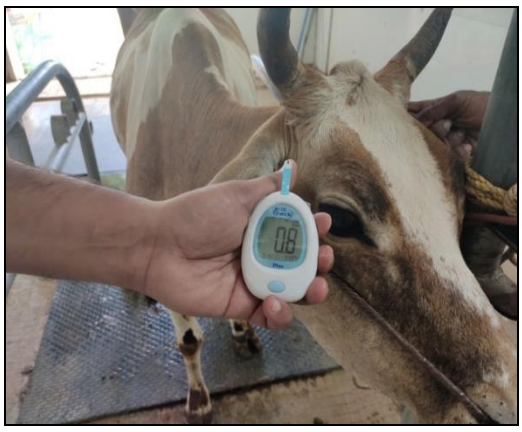

Fig 4: Estimation of Blood ketones using BHBcheck Plus ketonemeter

Conclusion

Based on the NEFA and blood BHBA, the prevalence of subclinical ketosis in postpartum anestrum animals is found to be 80 percent among the sample animals. However, NEFA among the control and postpartum anestrum cows does not differ in a remarkable manner. Hence using bovine specific ketone meter for diagnosing subclinical ketosis especially in the postpartum period will help in managing the nutritive status and prevent prolonged ovarian acyclicity.

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