The Pharma Innovation Journal 2023; SP-12(7): 198-203

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(7): 198-203
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www.thepharmajournal.com
Received: 08-05-2023
Accepted: 12-06-2023

Enbavelan PA
Assistant Professor, Department of Veterinary Medicine, VCRI, Salem, Tamil Nadu, India

Kavitha S
Professor and Head, Department of Veterinary Clinical Medicine, Madras Veterinary College, Chennai, Tamil Nadu, India

Annal Villi R
Professor and Head, Veterinary University Training and Research Centre, Krishnagiri, Tamil Nadu, India

Vairamuthu S
Professor and Head, Centralized Clinical Laboratory, Madras Veterinary College, Chennai, Tamil Nadu, India

Selvaraj P
Professor and Head, Veterinary University Peripheral Hospital, TANUVAS, Madhavaram Milk Colony, Chennai, Tamil Nadu, India

Corresponding Author:
Enbavelan PA
Assistant Professor, Department of Veterinary Medicine, VCRI, Salem, Tamil Nadu, India

Cow-side test for early detection of sub clinical ketosis in post-partum period of dairy cows

Enbavelan PA, Kavitha S, Annal Villi R, Vairamuthu S and Selvaraj P

Abstract
Sub-Clinical Ketosis is a common metabolic disorder frequently observed in dairy cows during the early lactation period. After calving, the milk production of dairy cows increases rapidly, but the nutrient intake cannot meet the demand for milk production, forming a negative energy balance (NEB). The NEB leads to a large amount of body fat mobilization and consequently the elevated production of ketones, which have an increased risk of developing metabolic diseases such as Sub-clinical ketosis, Clinical ketosis and fatty liver. Subclinical ketosis (SCK) in dairy cattle is an excess level of circulating ketone bodies in the absence of clinical signs of ketosis. Calved animals (0^{th}, 15^{th}, 30^{th}, 45^{th} and 60^{th} day) from organised and unorganised dairy farms of villages in and around Krishnagiri district of Tamil Nadu from the period February 2021 to May 2023 total 211 post-partum dairy animals were subjected to determination of blood beta hydroxybutyric acid (BHBA) level by means of a portable blood ketone and glucose monitoring system, qualitative urinalysis using urine dip stick and milk BHB dip stick and Milk Fat: Protein ratio by inline milk analyser. Usually, detection of SCK is carried out by testing the ketone concentrations in blood, urine, and milk. SCK is the pre-clinical form of ketosis, if animals are in clinical ketosis even though successful treatment for clinical ketosis carried out after recovery not able to bring the animal to normal production. Hence, the purpose of this study was to evaluate the rapid and reliable cow side test for early detection of subclinical ketosis (SCK) in dairy cows of early lactation period and to determine the association of onset of SCK by blood β-hydroxybutyrate (BHBA) concentration (Ketometer), BHB in milk, Fat: Protein ratio, Acetoacetate in urine and glucometer. Those animals with beta hydroxybutyric acid level by bovine specific ketometer Quick Vet RAPID (Ubio Biotechnology Systems Pvt. Ltd., Kochi) is between > 1.2 mmol/L and < 1.4 mmol/L were classified as sub clinical ketosis and high prevalence was noticed on 15^{th} day of lactation 50.70% (36/71) followed by 30^{th} day 38.02% (27/71) followed by 45^{th} day 7% (5/71), 0^{th} day 2.8% (2/71) and 60^{th} day 1.4% (1/71).

Keywords: Metabolic disorder, β-hydroxybutyrate (BHBA), Sub clinical ketosis, milk fat: protein ratio, negative energy balance

Introduction
Subclinical ketosis (SCK) is defined as condition with high concentrations of ketone bodies in blood without exhibiting clinical signs. The condition has high economic significance as it led to greater losses than clinical ketosis in addition to predisposing the animal to other conditions like abomasal displacement, mastitis etc., (Suthar *et al.* 2013) [21]. Often the condition is left undiagnosed and untreated as the clinical signs are not prominent and hence will not be noticed by the farmers. Among the diagnostic tests assessment of serum BHBA is gold standard with thresh hold levels > 1.2 mmol/L. SCK has major production impact even after successful treatment, so early detection by rapid field tools like BHBA, Milk Fat: protein ratio and Milk BHB would help famers from severe economic loss. Hence, the present study was conducted to study subclinical ketosis among crossbred cows in and around Krishnagiri, Tamil Nadu to explore the importance of early detection of SCK by Cow-side tests.

Materials and Methods
Selection of Animals
The study was conducted over a period of two years i.e., February 2021 to May 2023 in organized and unorganized dairy farms present in and around Krishnagiri, Tamil Nadu. Cows in their post calving period, belonging to different breeds were included in the study. All the cows were thoroughly examined for presence of any other ailments and those found positive were excluded from the study. A total of 211 animals in which 138 Holstein Friesian cross breed cows and Jersey cross bred cows are belongs to 1^{st} calving to 5^{th} calving were included in our study and subjected for determination of BHBA, Milk Fat: protein ratio and Milk BHB

by cow-side tests for early detection of SCK.

Cow-Side test

1. Estimation of Concentration of BHBA

The blood BHBA concentration was determine by using a portable blood Ketone monitoring system –Quick Vet RAPID (Fig. 1). The Tail vein was punctured with a sterile 23G needle and the ketone meter attached with blood ketone strip was directed towards the drop of blood. Sufficient quantity of blood droplet was absorbed at the tip of the strip by capillary action and wait for 5 to 10 seconds the blood BHBA concentration was displayed on to the digital meter (Fig. 4). Animals with blood BHBA concentration ranging between 1.2 – 1.4 mmol/L were considered to be SCK in the transition dairy period of animals (Jeppesen *et al*., 2006, Iwersen *et al*., 2009; Voyvoda and Erdogan, 2010; Panousis *et al*., 2012; Dore *et al*., 2013) [14, 13, 23, 19, 4].

2. Estimation of Milk BHB

Milk BHB concentration was determined using Porta BHB Milk Ketone Test (Strip test) (Fig. 2). Dip the test strip into a vial of fresh milk or strip directly onto the test. Just one minute later, compare the color change to the color chart. The cut-off concentration of BHB in milk was set at ≥0.080 mmol/L (Ježek *et al*., 2017) [15].

3. Estimation of Fat: Protein ratio

Fat: protein ratio was estimated using Eko Milk Ultra Pro machine (Fig. 3 & 4), Fat: protein ratio 1.4:1 was taken as subclinical ketosis and those with >1.4:1 were considered as clinical ketosis (Guliński. 2021) [11].

4. Estimation of Urine Ketone bodies

The ketone strips (Ketostix, Bayern Corporation, Elkhart, IN) (Fig. No. 5) was used to urine ketone bodies a dipstick containing the salt nitroprusside, which becomes pink in the presence of acetoacetate (AcAc), thus estimating the amount of AcAc in mg/dL. The color intensity varies with the amount of AcAc in urine (Galvão *et al*., 2012) [9]. Tests were performed as described by the manufacturer on spontaneous urination or urination induced by manual stimulation of escutcheon (area below the vulva). The Ketostix diagnostic test are read in five categories: (1) negative (0 mg/dL), (2) trace (5 mg/dL), (3) small (15 mg/dL), (4) moderate (40 mg/dL), and (5) large (greater than 80 mg/dL of AcAc) presence of ketone bodies.

5. Estimation of Glucose

The glucose level was estimated by using bovine specific glucometer (Ubio Rapid Quick vet). The Tail vein was punctured with a sterile 23G needle and the ketone meter attached with blood ketone strip was directed towards the drop of blood. Sufficient quantity of blood droplet was absorbed at the tip of the strip by capillary action and wait for 5 to 10 seconds the blood glucose concentration was displayed on to the digital meter (Voyvoda and Erdogan 2010) [23] (Fig. 6).

Fig 1: Blood BHBA (Quick Vet RAPID)

Fig 2: Milk BHB strip test

Fig 3: Milk analyzer for Fat and Protein

Fig 4: Fat: Protein Ratio

Fig 5: Urine Strip test (Acetoacetate)

Fig 6: Bovine Glucometer (Blood Glucose)

Fig 7: Post-partum animals with calf

Results

A total of 211 cows in their post calving period was screened for subclinical ketosis from 0th day to 60th day of lactation. Out of 211 cases, 138 were Holstein Friesian and 73 were Jersey cross breed, among the 138 Holstein Friesian cows screened 45 (32.60%) were found to be positive for SCK while 26 (35.61%) Jersey cross breeds were positive with an overall prevalence of 33.64% (71/211). Lactation wise prevalence was studied which revealed higher incidence in third lactation 47.88% (34/71) followed by fourth lactation 39.43% (28/71) and second lactation 12.67% (9/71). No animal was found to be affected with SCK during their first lactation period.

Blood BHBA screening was done for every 15 days and high prevalence was noticed on 15th day of lactation 50.70% (36/71) followed by 30th day 38.02% (27/71) followed by 45th day 7% (5/71), 0th day 2.8% (2/71) and 60th day 1.4% (1/71).

Milk BHB estimation was done and levels between 100 to

200 micromol/L was considered to be positive for subclinical ketosis while levels greater than 200 micromol/L was considered clinical ketosis. In the present study day wise correlation revealed 22 animals positive for SCK on 15th day, 12 animals on 30th day and no animals were found to be positive on milk test on 0th day, 45th day and 60th day. Milk fat: protein ratio estimation revealed higher prevalence on 15th day of lactation 40.84% (29/71) followed by 30th day of lactation 29.57% (21/71), 0th day of lactation 11.26% (8/71), 45th day of lactation 5.63% (4/71) and 60th day of lactation 1.40% (1/71).

Urine analysis of BHB was semi quantitatively estimated using urine strip test for acetoacetate with grading depicted in fig.5. In the study higher incidence of sub clinical ketosis was noticed on 15th day of lactation 42.25% (30/71) followed by 30th day 33.80% (24/71), 45th day 31.12% (15/71), 0th day 2.81% (2/71) and 60th day 1.40% (1/71).

Glucose estimated by using glucometer to find hypoglycemic condition in SCK was not noticed on 15th day of lactation 47.88% (34/71) followed by 30th day 38.02% (27/71) followed by 45th day 4.22% (3/71) followed by 60th day 1.40% (1/71) and 0th day 2.81% (2/71) of lactation.

On comparing different days during lactation, high prevalence of SCK was noticed on 15th day on all cow side tests as well as followed by 30th day of lactation. Prevalence on other days of study was less compared to 15th and 30th days. Of all the tests done, blood BHBA has highest sensitivity in diagnosing SCK followed by urine acetoacetate, Milk fat: protein ratio and milk BHB.

Table 1: Comparison of different diagnostic tests for detecting SCK at different time intervals

Parameters	0th day	15th day	30th day	45th day	60th day
Blood BHBA	2	36	27	5	1
Milk BHBA	-	22	12	-	-
Milk Fat: Protein	-	29	21	4	1
Urine acetoacetate	2	30	24	15	1
Glucometer	2	34	27	3	1

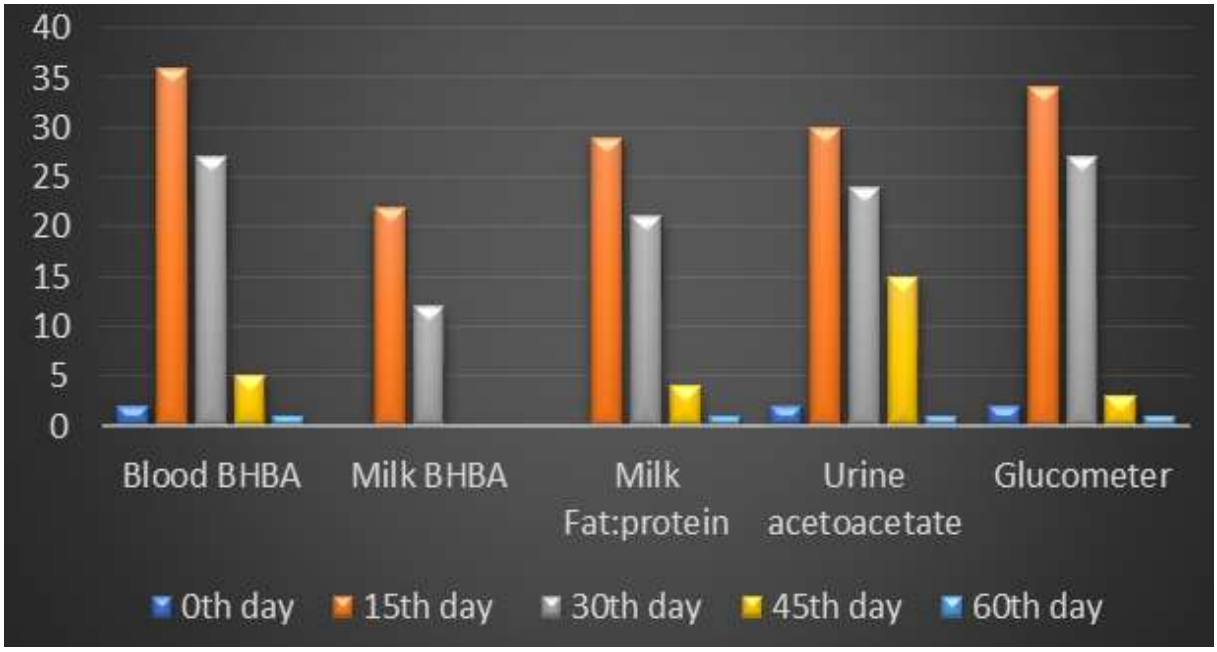

Fig 8: Cowside test lactation day-wise comparision in post-partum dairy cows

In this study on 15th day of post-partum period found that most of the animals showed positive to SCK using cowside test such Blood BHBA (Ketometer), Milk BHB strip, Milk Fat: Protein ratio (Milk analyser), Urine acetoacetate strip and Glucose (Glucometer) represented as 50.7%, 30.98%, 40.84%, 42.25% and 47.88% respectively.

Discussion

In the present study higher prevalence of SCK was noticed in Holstein Friesian when compared with jersey cows. It is well known fact that Holstein Friesian are high yielders compared to jerseys, however the milk from the later has high amount of fat and protein (Lei and Simoes, 2021) [18] and also its high genetic susceptibility affected by numerous regions across the genome (Gaddis *et al*, 2018) [8] and maximum use of this germplasm in crossbreeding program for accelerating milk production (Venkateswarulu and Rao, 1993) [22].

Lactation wise study showed higher prevalence in third and fourth lactations compared to earlier ones. The largest occurrence was found in the third and fourth lactation, which was because of the animal's high output that caused a severe lactational glucose drain and increased sensitivity to sub clinical ketosis. The animal's reduced level of production during the first lactation was the cause of the lower prevalence (Krishna K S *et al*. 2014) [16] and possible reason for that might be possible reason for that might be the active homeostatic mechanisms in young dairy animals to cope up with negative energy balance during early lactation period. Similar to the present findings, a number of research workers recorded that prevalence of Sub clinical ketosis increased with age and peak prevalence was observed between third and fifth lactation.

Blood BHBA estimation is the gold standard for the early diagnosis of SCK and was compared with other cow side and

laboratory diagnostic methods. Seventy one cows were found positive on the basis of postpartum blood BHBA concentration of 1.2 - 1.4 mmol/L and considered as subclinical ketosis which were in accordance to (LeBlanc, 2010; Rollin *et al*., 2010; Suthar *et al*., 2013; Compton *et al*., 2014; Garro *et al*., 2014) [17, 20, 21, 3, 10] which may be due to high concentration of circulating ketone bodies, mainly BHB during extreme negative energy balance in early lactating dairy cows (Duffield *et al*., 2009) [6].

Second to blood BHBA, Urine acetoacetate was found to be sensitive in identifying the positive cases. It was well established that urine acetoacetate levels and blood BHBA levels are directly correlated and hence the urine strip test can be well utilised for diagnosing sub-clinical ketosis in cattle (Faruk *et al*., 2020 and Alahi *et al*. 2022) [7]. The key finding of this study is that no difference exists in BHBA concentration between cows that had a trace or small in the Ketostix reading. Using trace to diagnose cows with SCK resulted in good sensitivity and specificity in accordance with Galvão *et al*., 2012 [9]. Our findings indicate to treat any cow with a trace reading.

Milk fat

Protein ratio was also done to evaluate its efficacy and was found to have almost similar efficacy as of urine acetoacetate. The amount of fat: protein reflects the energy status of the animal and during high production states there will be an increased fat and reduced protein (Lei and Simoes, 2021) [18] and is a potential indicator of energy deficit in these lactating cows (Heuer *et al*. 1999). Further this changes attributed by lipolysis from falling of IGF1 and leptin, insulin signalling blockage which in turns increases milk fat content and decreases protein in milk (Guliński, 2021) [11].

Milk BHBA was able to diagnose 61.1% of positives as diagnosed by blood BHBA during the peak incidence period of 15th day. This finding was in agreement with that of Faruk *et al*. (2020) [7] who reported an agreement of 29.6 % to 40.7 % of between urine and milk ketones estimation basing on the kit utilised in SCK animals. The milk test's reduced sensitivity also means that it is less able to identify SCK animals, which are actually positive for SCK. Because milk contains many noisy substances, such as proteins, lipids, minerals, etc., a real SCK may occasionally be seen as a normal result in a milk test (Cheruiyot *et al*. 2018) [2].

The highest prevalence of SCK was found during second week of lactation (15th day) the same is attributed by Duffield *et al*., 2003 [5].

Conclusion

In conclusion, the results of this study showed that the handheld meter is rapid and reliable for measuring blood BHBA concentrations, glucose and can be used as a cowside test for early detection of SCK. Milk fat: Protein ratio is also easily tested in milk collection unit to early detection of SCK by the farmers weekly once or monthly once.

Hence determination of blood βhydroxybutyric acid (BHBA), and glucose concentration using portable blood ketone / glucose meter and qualitative and semi quantitative milk and urinalysis using urine and milk dip stick for the presence of ketone bodies are the reliable indicators in the early diagnosis of SCK under field conditions.

Especially on day 15th of lactation would be better period to detect SCK using cowside test, among this BHBA can a benchmark for early detection of SCK. Strip test is more useful to early detection of SCK in field level investigation and herd level monitoring for proper treatment and management to prevent economic losses to farmers and dairy industry.

Reference

1. Alahi MR, Khan MA, Qamar AG, Jabbar MA, Iqbal A, Arshad M, *et al*. Comparative evaluation of ketone bodies in blood and urine for the detection of subclinical ketosis in postpartum buffaloes. Pakistan Journal of Science. 2022;74(4):403-411.
2. Cheruiyot EK, Bett RC, Amimo JO, Mujibi FD. Milk composition for admixed dairy cattle in Tanzania. Frontiers in Genetics. 2018;9:142.
3. Compton CW, McDougall S, Young L, Bryan MA. Prevalence of subclinical ketosis in mainly pasture-grazed dairy cows in New Zealand in early lactation. N. Z. Vet. J. 2014;62:30–37.
4. Dore V, Dubuc J, Belanger AM, Buczinski S. Short communication: Evaluation of the accuracy of an electronic on-farm test to quantify blood β-hydroxybutyrate concentration in dairy goats. J. Dairy Sci. 2013;96:4505-4507.
5. Duffield TF, LeBlanc S, Bagg R, Leslie K, Hag JT, Dick P. Effect of a monensin-controlled release capsule on metabolic parameters in transition cows. J Dairy Sci. 2003;86:1171–1176.
6. Duffield TF, Lissemore KD, McBride BW, Leslie KE. Impact of hyperketonemia in early lactation dairy cows on health and production. J Dairy Sci. 2009;92:571-580.
7. Faruk MS, Park B, Ha S, Lee SS, Mamuad LL, Cho Y. Comparative study on different field tests of ketosis using blood, milk, and urine in dairy cattle. Veterinární medicína. 2020;65(5):199-206.
8. Gaddis KP, Megonigal Jr JH, Clay JS, Wolfe CW. Genome-wide association study for ketosis in US Jerseys using producer-recorded data. Journal of dairy science. 2018;101(1):413-424.
9. Galvão KN, Neto AV, Peña G, Bittar J, Ibarbia L. Comparing the Urine Ketone Strip Test and the Handheld Ketone Meter to Diagnose Ketosis in Early Lactation Dairy Cows: VM186/VM186, 10/2012. EDIS. 2012. p. 1-4.
10. Garro CJ, Mian L, Cobos Roldán M. Subclinical ketosis in dairy cows: prevalence and risk factors in grazing production system. J Anim. Physiol. Anim. Nutr. (Berl). 2014;98:838–844.
11. Guliński P. Ketone bodies – causes and effects of their increased presence in cows' body fluids: A review, Veterinary World. 2021;14(6):1492-1503.
12. Heuer C, Schukken YH, Dobbelaar P. Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. Journal of dairy science. 1999;82(2):295-304.
13. Iwersen M, Falkenberg U, Voigtsberger R, Forderung D Heuwieser W. Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. Journal of Dairy Science. 2009;92(6):2618-2624.
14. Jeppesen RJ, Enemark MD, Enevoldsen C. Ketone body measurement in dairy cows Proc. 24th World Buiatrics Congress, Nice, France. World Assoc., Buiatrics, Vienna, Austria. 2006.
15. Ježek J, Cincović MR, Nemec M, Belić B, Djoković R,

Klinkon M, *et al*. Beta-hydroxybutyrate in milk as screening test for subclinical ketosis in dairy cows. Pol J Vet Sci. 2017;20(3):507-512.
16. Krishna KS, Suresh K, Sreenu M, Sundar NS. Prevalence of subclinical hypocalcaemia and subclinical ketosis in buffaloes. Buffalo Bulletin. 2014;33(1):107-110.
17. LeBlanc SJ. Monitoring metabolic health of dairy cattle in the transition period. J Reprod. Develop. 2010;56:S29-S35.
18. Lei MAC, Simões J. Invited review: ketosis diagnosis and monitoring in high-producing dairy cows. Dairy. 2021;2(2):303-325.
19. Panousis N, Brozos C, Karagiannis I. Evaluation of precision Xceed meter for on-site monitoring of blood B-hydroxybutyric acid and glucose concentrations in dairy sheep. Res. Vet. Sci. 2012;93:435-439,
20. Rollin E, Berghaus RD, Rapnicki P, Godden SM, Overton MW. The effect of injectable butaphosphan and cyanocobalamin on postpartum serum beta-hydroxybutyrate, calcium, and phosphorus concentrations in dairy cattle. J Dairy Sci. 2010;93:978-987.
21. Suthar VS, Canelas-Raposo J, Deniz A, Heuwieser W. Prevalence of subclinical ketosis and relationships with postpartum diseases in European dairy cows. J Dairy Sci. 2013;96(5):2925-38.
22. Venkateswarulu K, Rao DST. Subclinical ketosis in crossbred cattle. Cheiron. 1993;22:116-119.
23. Voyvoda H, Erdogan H. Use of a hand-held meter for detecting subclinical ketosis in dairy cows. Research in veterinary science. 2010;89(3):344-351.