The Pharma Innovation Journal 2018; 7(7): 937-939

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2018; 7(7): 937-939

www.thepharmajournal.com
Received: 04-05-2018
Accepted: 05-06-2018

Yogalakshmi Sabapathy
Translational Research Platform for Veterinary Biologicals, Madhavaram Milk Colony, Chennai, Tamil Nadu, India

Alagu Maruthanayagam
Translational Research Platform for Veterinary Biologicals, Madhavaram Milk Colony, Chennai, Tamil Nadu, India

Umadevi Subramanian
Translational Research Platform for Veterinary Biologicals, Madhavaram Milk Colony, Chennai, Tamil Nadu, India

Dhinakar Raj Gopal
Translational Research Platform for Veterinary Biologicals, Madhavaram Milk Colony, Chennai, Tamil Nadu, India

Correspondence
Dhinakar Raj Gopal
Translational Research Platform for Veterinary Biologicals, Madhavaram Milk Colony, Chennai, Tamil Nadu, India

Validation and field application of in-house developed ketosis diagnosis kit

Yogalakshmi Sabapathy, Alagu Maruthanayagam, Umadevi Subramanian and Dhinakar Raj Gopal

Abstract
Ketosis is a common metabolic disorder of dairy cows that occurs mainly due to negative energy balance. The prevalence of ketosis is very high in early lactation period and it occurs even in best managed farms. In order to have a constant monitoring to manage the condition, a low cost cow-side test is needed. Despite many laboratory methods available, due to the cost affairs, those methods cannot be applied for routine monitoring. At TRPVB, a diagnostic kit (KetoCheck) that detects β-hydroxy butyrate (BHB) in urine was developed. In the present study, the sensitivity and specificity of Ketocheck was estimated to demonstrate its effectiveness as a cow-side test. In this study, the performance of Keto Check kit was compared with the commercially available kits such as SD Urocolor (Standard Diagnostics, Inc. USA) and FreeStyle Optium H (Abbott Laboratories, USA) using bovine urine samples. Lactating cows (n=54) were screened using the above mentioned diagnostic methods and the results were compared. Out of 54 samples screened selectively, thirty eight samples (70.4%) were positive in Ketocheck kit, thirty seven (68.5%) in FreeStyle Optium H meter and thirty eight (70.4%) for SD Urocolor. The positive samples were also confirmed clinically as ketosis. The diagnostic sensitivity and specificity of Ketocheck kit was found to be 94.6% and 82.4% respectively compared to FreeStyle Optium H meter (Gold standard method). The observed correlation between Ketocheck kit and FreeStyle Optium H meter demonstrates the effectiveness of Ketocheck kit as an affordable cow-side test for BHB detection in urine samples for routine monitoring of herds. In addition, the cost per assay in the developed kit was reduced by around 80% of the costs involved in commercial tests.

Keywords: Ketosis, ketocheck, cow-side test, BHB

1. Introduction

The dairy farming plays a major role in Indian economy being the largest producer of milk worldwide. Hence any economic loss to dairy industry has a direct impact on Indian economy. Presently, researchers and farmers are focusing towards cross breeding practices to increase the milk production. But these practices result in increased susceptibility to various metabolic disorders like Laminitis, Ketosis, Fatty liver, Milk fever, retained placenta, Left displaced abomasums, downer cow, metritis and bloat (Sharma, 2010; Raboisson *et al*., 2014; McArt *et al*., 2012) [12, 9, 6]. The occurrence of these metabolic disorders is mainly due to lack of knowledge on nutrient and energy supplement to dairy cows according to their milk production. Moreover these metabolic disorders lead to decreased milk production, high culling rate, impaired fertility etc. Hence much attention has to be given to these metabolic disorders diagnosis and treatment. Ketosis is a common metabolic disorder of dairy cows which occurs during early lactation period due to negative energy balance (NEB).The dramatic increase in energy requirements for fetal growth and milk synthesis leads to NEB (Herdt, 2000; Ospina *et al*., 2013; Sundrum, 2015) [3, 7, 13]. During NEB and low glucose availability, the dairy cows meet their energy requirements by lipolysis, which utilize fats for energy demand (Rukkwamsuk *et al*., 1999) [10].

Ketosis is classified into subclinical ketosis and clinical ketosis on the basis of blood BHB concentration. Clinical ketosis is characterized by an increase in blood, urine, or milk ketone bodies in association with other visible signs such as inappetence, rapid weight loss and decreased milk production (Duffield, 2000; Gordon *et al*., 2013) [1, 2]. The main three ketone bodies are acetone, acetoacetate and BHB. Among them, the major ketone body is BHB which constitutes about 78%, whereas acetoacetate and acetone are 20% and 2% respectively. The threshold value for SCK and CK is 1.2-2.9 mmol/L and ≥3mmol/L of blood BHB respectively (McArt *et al*., 2012) [6].

For the diagnosis of ketosis, many tests have been developed that detect ketone bodies in blood, milk as well as in urine samples. The quantification of ketone bodies requires special laboratory equipment, while semi quantitative tests (cow side tests) are easy and no need of any laboratory equipment and laboratory technician. It was reported that the urine ketone body concentrations are about four times higher than blood concentrations, while milk BHB concentrations are one-eighth and milk acetoacetate concentrations are about half that of blood concentrations (Schultz, 1968; LeBlanc, 2010; Weng *et al*., 2015) [11, 4, 15]. At TRPVB, a simple method to detect ketosis was developed and this in-house developed ketosis diagnosis kit was named as ketocheck. The ketocheck is a colorimetric method for the measurement of urine BHB in the urine samples. Compared to milk and blood, urine excretes more amounts of ketone bodies. Hence early detection of ketosis is possible using this kit. Moreover it is a cow side test, which is essential for constant monitoring. Hence, the objective of this study was to determine the diagnostic performance of the in-house developed ketocheck kit for use in dairy cattle.

2. Materials and methods

The urine samples were collected from local farms, veterinary clinics, Puduvazhal, Ponneri and Madras veterinary college, Chennai to check the performance of the kit and for field validation. The assay works under the principle that the chemicals in the reagent specifically reacts with the ketone bodies present in the urine samples. The instant color development in the reaction can be compared with the color chart to predict the severity of the ketosis condition. The developed assay was tested for its sensitivity and specificity. For this purpose, the urine samples were analysed using two commercial tests such as SD Urocolor and FreeStyle Optium H. SD Urocolor is a strip method that detects blood, bilirubin, urobilinogen, ketone, protein, nitrite, glucose, pH, specific gravity, leucocytes and ascorbic acid in urine sample. FreeStyle Optium H is a hand-held ketone meter that detects blood BHB.

3. Results and discussion

Ketosis is mainly due to negative energy balance which arises during lactation period because of the imbalance between energy supplied and energy required. The energy requirement is more because of milk synthesis (Wankhade *et al*., 2017) [14]. Ketosis requires constant monitoring and hence many cowside tests were available. Most of the cowside tests detect either acetoacetate or acetone which is unstable ketone bodies. For example, the Rothera's test can detect acetoacetate and acetone but not BHB. The cowside tests that detect BHB are enzymatic methods leading to high cost. Hence in order to overcome all these disadvantages, a low cost chemical method that detects BHB is developed and named as 'ketocheck'.

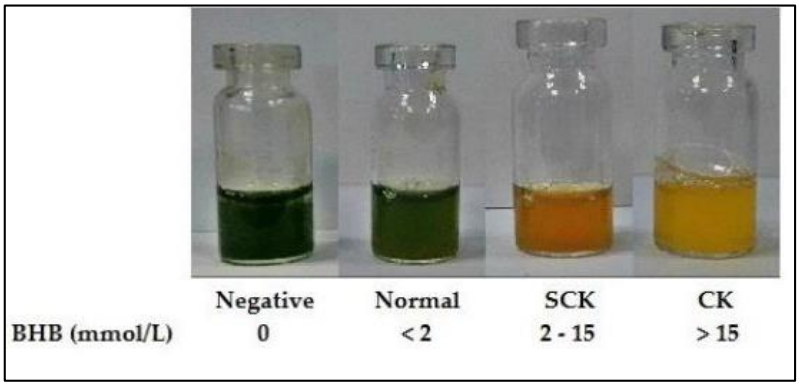

Fig: The colour range for BHB levels in ketocheck kit

To check the kit performance, urine samples were analyzed using ketocheck and SD Urocolor. The color developed for ketocheck kit was read at 620nm using multimode reader. The absorbance value less than 1.3 was considered significant for the ketosis. The random sampling was done for 154 samples. Forty two of the 154 cows (27.3%) are positive for ketocheck kit. The SD Urocolor test was positive for thirty seven samples out of 154 (24.03%). Out of fifty four clinical samples, thirty eight were shown to be positive for SD Urocolor and thirty seven were shown to be positive for BHB meter. Thirty eight samples were found to be positive for ketocheck kit. The gold standard method for ketosis diagnosis is measurement of blood BHB level (Pineda and Cardoso, 2015). Hence, in the present study the ketocheck kit was compared with BHB meter. Sensitivity = (35/35+2) x 100 = 94.6% Specificity = (14/14+3) x 100 = 82.4%. The normal ketone level in blood is < 0.5mmol/L. The ketone level for SCK and CK is 0.5- 2.8mmol/L and ≥ 2.9mmol/L respectively. On comparing with FreeStyle BHB meter, the ketocheck kit ketone level of urine sample for normal, SCK and CK is < 2 mmol/L, 2-15mmol/L and > 15mmol/L respectively. The Pearson's correlation coefficient of ketocheck kit with FreeStyle BHB meter and SD Urocolor is 0.99 and 0.86 respectively. The coefficient of 0.99 indicates strong relationship of ketocheck kit with FreeStyle BHB meter. The number of clinical samples tested in this study was less because of the difficulty in collecting urine samples. Further research is needed to make the ketocheck kit suitable for milk samples.

In this study, 54 clinical samples were analysed and the diagnostic performance of ketocheck kit was compared with SD Urocolor and FreeStyle BHB meter. The gold standard for ketosis diagnosis is blood BHB level which is measured using FreeStyle BHB meter. Out of 54 clinical samples (37 positive/ 17 negative by FreeStyle BHB meter) the ketocheck kit was able to detect 35 positive and 14 negative. Three samples were detected as false positive and two as false negative. The diagnostic sensitivity and specificity was calculated as 94.6% and 82.4% respectively. The positive predictive value of the ketocheck kit was 92.1% and the negative predictive value was 87.5%. On a note, the ketocheck kit displayed a positive result even the clinicians found it as a negative for ketosis.

This may be because of the animals had other clinical signs such as anaplasma, fatty liver, etc.

In conclusion, the ketocheck kit is a useful cowside ketone test for detection of SCK and CK using urine samples. Sensitivity and specificity of ketocheck kit was found to be high when compared with SD Urocolor. Moreover, the ketocheck kit is a low cost, non-invasive method for detecting BHB on comparison with commercial methods. Hence ketocheck kit can be used for constant monitoring of dairy cattle for ketosis.

4. References

1. Duffield T. Subclinical ketosis in lactating dairy cattle. Vet Clin North Am Food Anim Pract. 2000; 16(2):231-53.
2. Gordon JL, Leblanc SJ, Duffield TF. Ketosis treatment in lactating dairy cattle. Vet Clin North Am Food Anim Pract. 2013; 29(2):433-45.
3. Herdt TH. Ruminant adaptation to negative energy balance. Influences on the etiology of ketosis and fatty liver. Vet Clin North Am Food Anim Pract. 2000; 16(2):215-30.
4. LeBlanc S. Monitoring metabolic health of dairy cattle in the transition period. J Reprod Dev. 2010; 56:S29-35.
5. Li Y1, Xu C, Xia C, Zhang H, Sun L, Gao Y. Plasma metabolic profiling of dairy cows affected with clinical ketosis using LC/MS technology. Vet Q. 2014; 34(3):152-8.
6. McArt JAA, Nydam DV, Oetzel GR. Epidemiology of subclinical ketosis in early lactation dairy cattle. J Dairy Sci. 2012; 95(9):5056-5066.
7. Ospina PA, McArt JA, Overton TR, Stokol T, Nydam DV. Using nonesterified fatty acids and β-hydroxybutyrate concentrations during the transition period for herd-level monitoring of increased risk of disease and decreased reproductive and milking performance. Vet Clin North Am Food Anim Pract. 2013; 29(2):387-412.
8. Pineda A, Cardoso FC. Technical note: Validation of a handheld meter for measuring β-hydroxybutyrate concentrations in plasma and serum from dairy cows. J Dairy Sci. 2015; 98(12):8818-24.
9. Raboisson D, Mounié M, Maigné E. Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: a meta-analysis and review. J Dairy Sci. 2014; 97(12):7547-63.
10. Rukkwamsuk T, Kruip TA, Wensing T. Relationship between overfeeding and overconditioning in the dry period and the problems of high producing dairy cows during the postparturient period. Vet Q. 1999; 21(3):71-7.
11. Schultz LH. Ketosis in dairy cattle. J Dairy Sci. 1968; 51:1133.
12. Sharma N. Economically important production diseases of dairy animals. SMVD Dairy Year Book-2010. Published by Sarva Manav Vikash Samiti, Gurgoan, India, 2010, 47-65.
13. Sundrum A. Metabolic Disorders in the Transition Period Indicate that the Dairy Cows' Ability to Adapt is overstressed. Animals (Basel). 2015; 5(4):978-1020.
14. Wankhade PR, Manimaran A, Kumaresan A, Jeyakumar S, Ramesha KP, Sejian V *et al*. Metabolic and immunological changes in transition dairy cows: A review. Vet World. 2017; 10(11):1367-1377
15. Weng X, Zhao W, Neethirajan S, Duffield T. Microfluidic biosensor for β-Hydroxybutyrate (βHBA) determination of subclinical ketosis diagnosis. J Nanobiotechnology. 2015; 12:13:13.