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Evaluation of a Hand-Held Meter to Detect Subclinical Ketosis in Dairy Cows

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Abstract

The objective of this study was to evaluate the performance of a new cowside test in detecting sub-clinical ketosis (SCK) by comparing β-hydroxybutyrate (BHBA) concentrations obtained using a new hand-held meter, TNN (Yicheng Co., Beijing, China), with those obtained from the conventional laboratory method and to compare the accuracy of using blood, urine and milk samples for diagnostic purposes. TNN is based on an enzymatic electrochemical technique to detect SCK in dairy cows. 297 samples of blood, 97 samples of urine and 85 samples of milk from clinically healthy Holstein cows from d 1 to 58 post-partum were analyzed. The correlation coefficients for BHBA with TNN versus laboratory method were 0.98. Based on Bland-Altman plot, agreement between the two methods was good for BHBA. In this study, the TNN test had sensitivities of 91.4 and 96.8% at 1.2 and 1.4 mmol of BHBA/L, respectively of whole blood. While the specificities were 97.3% and 98.5%, at 1.2 and 1.4 mmol of BHBA/L, respectively. When compared with TNNthe sensitivities and specificities of urine and milk tests were lower. The sensitivities and specificities were 57 and 80% for milk tests, respectively, and 71% and 94% for urine tests, respectively, when 1.2 mmol/L of blood was defined as the threshold. Raising the threshold of laboratory method to 1.4 mmol/L, the sensitivities and specificities was 75 and 79% for milk tests, and 75 and 93% for urine tests, respectively. We concluded that TNN was a useful tool in diagnosis of SCK and blood tests were better than urine and milk tests.

Keywords: Subclinical ketosis; β-hydroxybutyrate; Blood

Introduction

Subclinical ketosis (SCK) is defined as an abnormal concentration of blood circulating ketone bodies without clinical signs of ketosis [1]. It is a common and important metabolic disease in high-lactating dairy cows which typically occurs in the first 60 d post-partum [2]. In the same period, the overall prevalence of subclinical ketosis ranges from 6.9 to 34% [3-7] with a peak observed within 14 d [8]. Sub clinical ketosis causes economic losses in the dairy industry as a result of impaired milk production and longer calving intervals [3,6,9-11]. Besides, cows with subclinical ketosis are at increased risk of developing cystic ovaries, clinical ketosis, and displaced abomasum [3,6,9-11]. In the light of these shortfalls, the cost of SCK per cow is estimated to be 78 dollars, whereas one case of clinical ketosis can induce economic losses of up to 145 [12,13]. It has been postulated that early diagnosis of individual cases of subclinical ketosis would permit for early treatment and may help mitigate further losses [14,12].

Elevated concentrations of ketone bodies [acetone (Ac), acetoacetate (AcAc), and BHBA] in blood, urine, milk, and other body fluids without any clinical signs are the main characteristic of SCK, and various thresholds have been used to define SCK [2,6,15,16]. The gold standard diagnostic test for SCK is measuring BHBA concentration in serum or plasma because it is stable [13,17,18]. Threshold values of either 1.2 mmol/L [19,20] or 1.4 mmol/L [6,12,16,17] of BHBA in serum or plasma have been recommended to distinguish between normal and cows suffering from SCK. Measuring serum concentration of BHBA is useful for examining individual cows, evaluating herd health and monitoring feeding management [21]. However, the quantitative determination of BHBA depends on special laboratory equipment and requires blood sampling, centrifugation, freezing of plasma or serum samples, and shipping of frozen samples to the laboratory [21]. These methods are thus neither convenient nor cost-effective for routine cowside diagnostic test in early detection of SCK [2]. Several rapid cowside tests have been developed to try and abate these inconveniences, reduce laboratory costs, and provide results immediately after sampling. However, certain limitations in their use exist, such as inadequate sensitivities for milk tests, suboptimal specificities for urine tests and the urine sampling requirements [22,23]. Oetzel [20] reported that cowside tests in blood BHBA had better sensitivity and specificity compared to urine and milk. Thus, blood ketone test methods that quantify BHBA have analytical, clinical and technical advantages over the cowside milk and urine tests in diagnosis or monitoring of SCK [2].

Hand-held meter tests for BHBA have been useful in diagnosis and monitoring of diabetic ketosis in humans because of its high sensitivity and specificity [24,25]. Few studies have shown that hand-held meters are useful in detecting subclinical ketosis of dairy cows [21,2]. Therefore, the objective of this study was to evaluate the performance of a new hand-held meter (TNN, Yicheng, Beijing,China) which is used to diagnose SCK in dairy cows by comparing its accuracy with laboratory methods for determining BHBA concentration in blood, milk and urine samples.

Materials and Methods

The experiment was conducted in accordance with the established standards outlined in the Guide for the Care and Use of Agriculture Animals in Agriculture Research and Teaching [9]. A total of 298 Holstein cows from three commercial farms (2 in Beijing and 1 in AnHui province, China) were included in this study. Based on clinical

examinations, all cows were healthy on the sampling days (d 1 to 58 post-partum). Sampling was carried out from September to October 2012.

The blood samples were collected from the coccygeal vessels using K3-EDTA-coated tubes before morning feeding (6.30 am to 7.30 am). Urine and milk were sampled on the same day. Blood, urine and milk were tested by cowside test immediately after sampling. Milk was tested with BHBA testing strips (Shanghai Bright, China). Urine was tested with URS (Urine Reagent Strip) strips (Nantong Diagnos Biotechnology Co., Ltd. Jiangsu, China). Blood was tested with TNN hand-held meter (Cheng, Beijing, China). The TNN device measures BHBA in whole blood by an enzyme-based electrochemical technique. When the blood sample is applied on the β-ketone test strip, the BHBA in the blood reacts with the chemical in the test strip producing a small electrical current. The current is measured by a sensor and the results are digitally displayed. The amount of current correlates with the amount of BHBA. According to the manufacturer, the results are shown within 30s and amount of BHBA that can be detected is given in the range of 0-6.0 mmol/L. After the BHBA test, the rest of the blood samples were centrifuged at 3000 g for 10 min and plasma was separated, stored in centrifugal tubes and stored at -20 pending further analysis. The plasma concentration of BHBA was measured using a D-3-hydroxybutyrate dehydrogenase enzymatic method (Hitachi 7020 auto-biochemistry, Randox Laboratories Ltd., Co. Antrim, UK). The enzymatic analysis was used as the laboratory reference method for validation purposes.

Data were analyzed using SPSS for Windows (Version 20.0). The significant differences between the means of BHBA in blood measured by the two methods was evaluated by Student's t-test for paired samples. The correlation coefficients (Pearson) were calculated between BHBA concentrations in plasma measured by the laboratory method and the values obtained by TNN device for the whole blood. Agreement between the two blood testing methods was verified using the Bland-Altman difference plot and Passing-Bablok regression using the laboratory methods as the comparison method. Significance was declared at $P \leq 0.05$.

Using plasma concentrations of BHBA as the standard for determination of SCK, the three cowside tests were evaluated by comparing their sensitivities, specificities, positive predictive values and negative predictive values.

Results

In this study, SCK was defined as plasma BHBA concentrations either ≥ 1.2 or ≥ 1.4 mmol/L. Of the 298 cows studied, 13.1 (39/298) and 11.4% (34/298) had SCK of ≥ 1.2 and ≥ 1.4 mmol BHBA/L, respectively in the whole blood measured using the hand-held device. On the other hand, laboratory method reported 11.7 and 10.4% for ≥ 1.2 and ≥ 1.4 mmol BHBA/L, respectively. Majority of SCK occurred within the first 3 wk of lactation, with few cases thereafter.

The means for BHBA concentrations measured using two different methods are shown in Table 1. Plasma concentrations of BHBA ranged from 0.1 to 5.8 mmol/L with the hand-held device and from 0.2 to 5.9 mmol/L using the laboratory method. There were no significant differences between the two methods (P=0.11). Comparison of BHBA data from the hand-held meter and the laboratory method revealed a high correlation between the two methods (r=0.98).

There was a linear relationship between the two methods in determining plasma concentration of BHBA (Figure 1). Examination of the difference plot showed a small positive bias of -0.02 mmol/L and the central 0.95 interval indicating a fair agreement between the applied methods for BHBA measurement (Figure 2). Thirty outliers (4.4%) were detected.

Parameter	Method (n=298)		
	Laboratory	Hand-held meter	P-Value
BHBA (mmol/L)	0.8231 ± 0.8148	0.8054 ± 0.7543	0.11

Table 1: Mean ± SD of BHBA concentrations measured using two different methods.

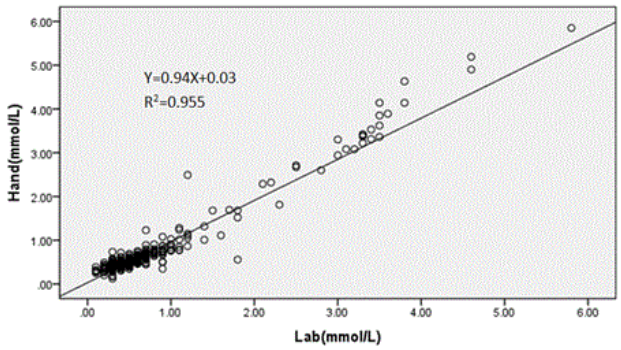

Figure 1: Passing-Bablok's regression between the laboratory method and the hand-held meter.

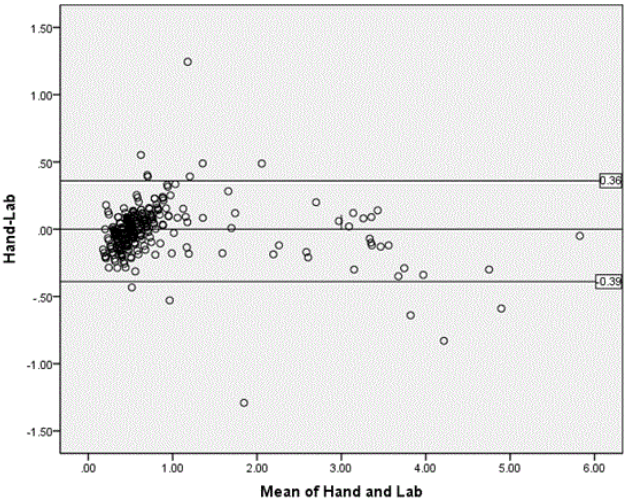

Figure 2: Bland-Altman's different plot of the laboratory method and the hand-held meter. Note: Hand-Lab means the difference between the concentration of BHBA measured by the laboratory method and the concentration of BHBA measured by the hand-held meter.

The test performance of the three cowside tests relative to plasma BHBA ≥ 1.2 mmol/L and ≥ 1.4 mmol/L for detection of SCK is shown

in Table 2. Compared to urine and milk tests, using blood sample test had a better accuracy. The sensitivities and specificities of urine and milk tests in measuring BHBA concentration were lower than those obtained by the hand-held meter. The sensitivity and specificity for hand-held meter at the threshold of 1.2 mmol/L BHBA were 91.4 and 97.3%, respectively. The sensitivity of the hand-held meter was higher in blood than both urine (71.4%) and milk (57.1%) tests (Table 2). Similarly, the specificity of hand-held meter was also higher. When the threshold of detection of SCK increased to 1.4 mmol/L BHBA, the sensitivity and specificity of the hand-held meter increased to 96.8 and 98.5%, respectively (Table 2).

Test	Plasma BHBA ≥ 1.2mmol as threshold				Plasama BHBA ≥ 1.4mmol as threshold			
	Sensitivity	Specificity	pV+	pV-	Sensitivity	Specificity	pV+	pV-
hand-held meter (blood	91.40%	97.30%	82.10%	98.80%	96.80%	98.50%	88.20%	99.60%
URS (urine)	71.40%	94.40%	50.00%	97.70%	75.00%	92.50%	30.00%	98.90%
Milk strip (milk)	57.10%	79.50%	20.00%	95.40%	75.00%	79.00%	15.00%	98.50%

Table 2: Performance of 3 cowside tests for detection subclinical ketosis.

Discussion

The threshold values for BHBA in serum or plasma of cows suffering from SCK has been defined either as 1.2 mmol/L or 1.4 mmol by different researchers [6,14,16,17,19]. The proportion of cows diagnosed with SCK was lower in this study compared to others [2,16,21,23] which showed that SCK was not a serious problem in the farms studied.

The BHBA concentrations were similar between the methods for the blood samples. The results were not consistent with the findings of Voyvoda and Erdogan [2] who reported that hand-held meter gave significantly higher values for plasma BHBA compared to laboratory methods. The correlation coefficient between the BHBA values obtained from the laboratory method compared to those obtained from the hand-held meter was 0.98 indicating that the hand-held method of testing SCK was as effective and accurate as the laboratory method. The results were in agreement with the findings of other researchers who reported that the coefficient of correlation between the laboratory methods and a hand-held meter in measuring plasma concentration for BHBA varied from 0.92 to 0.97 [2,21,26]. The agreement between the two methods was good since the bias was -0.02 which was lower than 0.036 reported by Voyvoda and Erdogan [2]. In the previous reports the percentages of outliers were between 1.3% [2] and 3.0% [2]. The similarity in outliers with those reported by lwersen [2] might be attributed to the number of animals sampled (298 vs. 196 in the current and Iwersen's studies, respectively). The lower percentage of Voyvoda and Erdogan [2] study could be due to fewer number of animals (78 cows) used. The high correlation and agreement between the two methods supports our hypothesis that the hand-held meter can be used as an alternative to the laboratory method in measuring plasma concentration of BHBA.

The sensitivities and specificities of urine and milk tests in measuring BHBA concentration were lower than those obtained by the hand-held meter using blood samples. This was in agreement with the results of Oetzel [22] who reported that the hand-held meter can be used as a cow-side blood test for detecting SCK because of its higher sensitivity and specificity compared to the available urine and milk cowside tests. The sensitivity and specificity at the threshold of 1.2 mmol/L of plasma BHBA for hand-held meter in the current study were higher than those reported by Voyvoda and Erdogan [2]. At a threshold of 1.4 mmol/L BHBA for SCK, the sensitivity and specificity were in agreement with previous studies in which hand-held meter results ranged from 85 to 100%, and from 97 to 100%, respectively [2,21,27].

Conclusion

The results of this study showed that TNN hand-held meter can be used as a rapid and useful tool for detecting SCK because of its high accuracy in detecting blood BHBA concentration. Furthermore, blood samples were better than milk and urine samples in diagnosis of SCK.

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References

1. Andersson L (1988) Subclinical ketosis in dairy cows. Metabolic diseases of ruminant livestock. Vet Clin North Am Food Anim Pract 4: 233-251.
2. Voyvoda H, Erdogan H (2010) Use of a hand-held meter for detecting subclinical ketosis in dairy cows. Res Vet Sci 89: 344-351.
3. Dohoo IR, Martin SW (1984) Subclinical ketosis: prevalence and associations with production and disease. Can J Comp Med 48: 1-5.
4. Andersson L, Emanuelson U (1985) An epidemiological study of hyperketonaemia in Swedish dairy cows; determinants and the relation to fertility. Prev Vet Med 3: 449-462.
5. Nielen M, Aarts MG, Jonkers AG, Wensing T, Schukken YH (1994) Evaluation of two cowside tests for the detection of subclinical ketosis in dairy cows. Can Vet J 35: 229-232.
6. Duffield TF (1997) Effects of a monensin controlled release capsule on energy metabolism, health, and production in lactating dairy cattle. D.V.Sc. Thesis, Univ. Guelph, Canada.
7. Duffield T (2006) Impact of subclinical ketosis on health and performance. In: Proceedings of the North American Veterinary Conference, Orlando, Florida 20: 13-16.
8. Duffield TF, Sandals D, Leslie KE, Lissemore K, McBride BW, et al. (1998) Efficacy of monensin for the prevention of subclinical ketosis in lactating dairy cows. J Dairy Sci 81: 2866-2873.
9. Fass (2010) Animal Science Guide for the Care and Use of Agricultural Animals in Research and Teaching. In: Francos G, Insler G, Dirksen G (eds) Routine testing for milk beta-hydroxybutyrate for the detection of subclinical ketosis in dairy cows. Bovine Pract 31: 61-64.
10. Girschewski H, Voigt J, Piatkowski B (1977) Investigations on the occurrence, detection and treatment of subclinical ketosis in high performance cows. Mh Vet Med 32: 201-203.

11. Gustafsson H, Emanuelson H (1996) Milk acetone concentration as an indicator of hyperketonemia in dairy cows-The critical value revised. J Anim Sci 63:183-188.
12. Geishauser T, Leslie K, Kelton D, Duffield TF (2001) Monitoring for subclinical ketosis in dairy herds. Compend Contin Educ Prac Vet 23: S65-S71.
13. Duffield T (2003) Minimizing subclinical metabolic diseases. In: Proceedings of Tri-State Dairy Nutrition Conference, Fort Wayne, pp: 1-14.
14. Enjalbert F, Nicot MC, Bayourthe C, Moncoulon R (2001) Ketone bodies in milk and blood of dairy cows: Relationship between concentrations and utilization for detection of subclinical ketosis. J Dairy Sci 84: 583-589.
15. Andersson L (1984) Detection, Occurrence, Causes and Effects of Hyperketonaemia in Swedish Dairy Cows. Skara, Sweden.
16. Geishauser T, Leslie K, Tenhag J, Bashiri A (2000) Evaluation of eight cowside ketone tests in milk for detection of subclinical ketosis in dairy cows. J Dairy Sci 83: 296-299.
17. Duffield T (2000) Subclinical ketosis in lactating dairy cattle. Vet Clin North Am Food Anim Pract 16: 231-253.
18. Herdt TH (2000) Variability characteristics and test selection in herd-level nutritional and metabolic profile testing: metabolic disorders of ruminants. Vet Clin North Am Food Anim Pract 16: 387-403.
19. Sakha M, Ameri M, Sharifi H, Taheri I (2007) Bovine subclinical ketosis in dairy herds in Iran. Vet Res Commun 31: 673-679.
20. Geishauser T, Leslie K, Kelton D, Duffield T (1998) Evaluation of five cowside tests for use with milk to detect subclinical ketosis in dairy cows. J Dairy Sci 81: 438-443.
21. Iwersen M, Falkenberg U, Voigtsberger R, Forderung D, Heuwieser W (2009) Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. J Dairy Sci 92: 2618-2624.
22. Oetzel GR (2004) Monitoring and testing dairy herds for metabolic disease. Vet Clin North Am Food Anim Pract 20: 651-674.
23. Carrier J, Stewart S, Godden S, Fetrow J, Rapnicki P (2004) Evaluation and use of three cowside tests for detection of subclinical ketosis in early postpartum cows. J Dairy Sci 87: 3725-3735.
24. Bektas F, Eray O, Sari R, Akbas H (2004) Point of care blood ketone testing of diabetic patients in the emergency department. Endocr Res 30: 395-402.
25. Ham MR, Okada P, White PC (2004) Bedside ketone determination in diabetic children with hyperglycemia and ketosis in the acute care setting. Pediatr Diabetes 5: 39-43.
26. Jeppesen, R, Enemark JMD, Enevoldsen C (2006) Ketone body measurement in dairy cows. In: Proceedings of the 24th World Buiatrics Congress, Nice, France, Ref. OS, pp: 43-52.
27. Oetzel GR, McGuirk S (2007) Cowside blood BHBA testing with a hand-held "ketometer" fact sheet-version, University of Wisconsin-Madison, School of Veterinary Medicine.