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Use of a hand-held meter for detecting subclinical ketosis in dairy cows

Huseyin Voyvoda *, Hasan Erdogan

Department of Internal Medicine, Faculty of Veterinary Medicine, Adnan Menderes University, PK 17, 09016 Aydin, Turkey

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ABSTRACT

The Optium Xceed is a new hand-held meter for determining blood β-hydroxybutyrate (BHBA) and glucose in human medicine. The objective of this study was to compare BHBA and glucose results obtained using the hand-held meter with those results made with a laboratory method and to evaluate its usefulness as a cowside test in the diagnosis of subclinical ketosis (SCK) in dairy cows. Seventy-eight blood samples from clinically healthy Holstein cows between 5 and 60 days post-calving were analysed. BHBA and glucose values were significantly higher with the hand-held meter versus laboratory methods. Correlation coefficients (r) for BHBA and glucose with the Optium Xceed versus laboratory methods were 0.97 and 0.63, respectively. Based on Bland–Altman plot and Passing–Bablok regression, agreement between two methods was good for BHBA but the agreement for glucose was only fair. When SCK was defined as plasma BHBA levels $\geqslant$1200 μmol/L, the sensitivity and specificity of the hand-held meter ketone testing in determining SCK were 85% and 94%, respectively. Raising the threshold of the laboratory method to $\geqslant$1400 μmol/L, the sensitivity and specificity incremented to 0.90 and 0.98, respectively.

In conclusion, the blood ketone-monitoring device can be used as a rapid and reliable diagnostic test to detect SCK under field conditions.

1. Introduction

Subclinical ketosis (SCK) is a common and important disease in high-producing dairy cows that occurs typically in the first 2 months after calving. Its prevalence in dairy herds ranges from 8.9% to 34% (Stöber, 2002; Duffield, 2006). SCK causes economic losses resulting from decreased milk production, increased risk of specific periparturient diseases (metritis, mastitis, displaced abomasum, and clinical ketosis), and impaired reproductive performance (Dohoo and Martin, 1984; Geishauser et al., 2000; Duffield, 2006; LeBlanc, 2006; Walsh et al., 2007). It has been reported that on average, 40% of dairy cows are affected by SCK at least once during lactation, whereas an average of 5% experience clinical ketosis (Geishauser et al., 2001; Duffield, 2003). When the negative impacts are considered, the cost of SCK per cow is estimated to be $78, whereas one case of clinical ketosis can cause a loss of $145 (Geishauser et al., 2001; Duffield, 2003). However, because the subclinical form is more prevalent, its cost at the herd level is much higher (Geishauser et al., 1998; Duffield, 2003). Therefore, identification of cows suffering from SCK in the immediate postpartum period at herd level is important to reduce the negative side effects of the disease.

Elevated concentrations of ketone bodies [acetone (Ac), acetoacetate (AcAc), and BHBA] in blood, urine, milk, and other body fluids without the signs that accompany the clinical form are main characteristic of SCK (Duffield, 2000, 2003; Stöber, 2002), while serum glucose concentration is variable (Herdt, 2000). Therefore, the measurement of ketone bodies in body fluid is the only efficient means of detecting SCK (Duffield, 2000; Stöber, 2002). Cowside urine and milk ketone tests are traditionally performed for diagnosing or monitoring of SCK but they have certain limitations in their use (Oetzel, 2004). Cowside urine and most milk ketone tests can quantitatively or semi-quantitatively detect AcAc and, to a lesser degree, Ac through nitroprusside reaction (Geishauser et al., 1998; Laffel, 1999). The predominant ketone body in ketosis, BHBA, is not detectable by these tests (Bruss, 1997; Laffel, 1999), expect for a semi-quantitative milk BHBA test (Geishauser et al., 1998). Therefore, they do not allow an accurate assessment of the complete ketone production status of the animal. Furthermore, measuring urine and milk ketone levels using dipstick tests are affected by several factors such as renal function, some drugs and substances (Bruss, 1997; Laffel, 1999), difficulty in urine collection (Osborne et al., 2002), contact time of urine with reagent (Carrier et al., 2004), user (Oetzel, 2004), mastitis and malfermented silages (Gravert, 1991; Gasteiner, 2000). Lastly, none of currently available cowside urine and milk tests have perfect sensitivity and specificity compared to blood BHBA (Oetzel, 2004; Rollin, 2006). Thus, blood ketone testing methods that quantify BHBA have analytical, technical and clinical advantages over cowside urine and milk tests for diagnosing or monitoring of SCK.

The gold standard diagnostic test for SCK is the measurement of BHBA in serum or plasma because of stability and predominant circulating ketone body (Dohoo and Martin, 1984; Duffield, 2000,

* Corresponding author. Tel.: +90 256 2470700/105; fax: +90 256 2470720.
E-mail address: hvoyvoda@adu.edu.tr (H. Voyvoda).

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2004; Herdt, 2000; Oetzel, 2004; LeBlanc, 2006), and it is useful for examining individual cows and evaluating herd health (Duffield, 2000; Herdt, 2000; Oetzel, 2004). Until a few years ago, BHBA concentration in serum or plasma has been measured using a quantitative enzymatic procedure. This method takes time, careful sample handling, special instrumentation and skilled laboratory personnel. Therefore, it is neither convenient nor cost-effective for use as a routine cowside diagnostic test for the early detection of SCK. Recently, Abbott Diabetes Care Ltd. has developed a hand-held meter (Optium Xceed™) that represents the latest generation of advanced biosensor technology and measures easily and rapidly both blood BHBA and blood glucose for monitoring and treating diabetes in humans (Anonymous, 2006a). To our knowledge, there is no other human glucometer that is also able to measure blood BHBA. Studies that evaluated human blood glucose and/or BHBA testing have shown good agreement between the Optium Xceed and reference laboratory methods (Byrne et al., 2000; Ronald, 2008). Moreover, the hand-held meter BHBA test was found to be more sensitive than urinary ketone tests for diagnosing and monitoring diabetic ketosis or diabetic ketoacidosis in multiple human studies (Bektas et al., 2004; Ham et al., 2004; Guerci et al., 2005; Noyes et al., 2007; Taboulet et al., 2007). As far as the authors are aware, there are a few reports about the application of this hand-held meter to cattle (Endecott et al., 2004; Jeppesen et al., 2006; Kupczyński and Cupok, 2007; Oetzel and McGuirk, 2007; Iwersen et al., 2009) and to dogs and cats (Hoenig et al., 2008). Overall, BHBA results of these studies are extremely encouraging in detecting SCK in cattle and in determining diabetic ketoacidosis in dogs and cats.

The objective of this study was to compare the accuracy of the Optium Xceed with a laboratory method for measurements of blood BHBA and glucose and to evaluate its usefulness as a cowside testing in the diagnosis of SCK in dairy cows.

2. Material and methods

A total of 78 Holstein cows (3–8 years old) from five commercial dairy herds in the vicinity of Aydin, Turkey, were included in the study. On the day of the herd visit, all cows from days 5 to 60 postpartum that were healthy, according to the farmers and clinical examination, were sampled. The cows had one to six lactations, with milk yield ranging from 22.0 to 36.5 kg/day during the study. Sampling was carried out during the winter housing period from December 2007 until March 2008. Ambient temperatures at animal pens were between 17 and 23 °C during the study period.

The Optium Xceed is designed for near-patient glucose and BHBA testing using capillary whole blood sample that does not contain anticoagulant (Anonymous, 2006a). The manufacturer claims that venous whole blood samples containing heparin or EDTA as anticoagulant can also be used for glucose and BHBA measurements within 30 min (Anonymous, 2005, 2006c). Based on validation of the glucose and β-ketone test-strips for venous samples, blood samples were collected into K_3-EDTA-coated blood tubes by jugular venepuncture from all examined cows. To avoid a likely peak in blood BHBA after a meal, the samples were taken 4–5 h after morning feeding (Eicher et al., 1998; Oetzel, 2004). An 10-μL aliquot of EDTA–whole blood was analysed immediately for BHBA and glucose concentrations using the Optium Xceed (Abbott Diabetes Care Ltd., Witney, UK). A sample was also separated for a haematocrit determination using microcapillary tube and a microcentrifuge (Nuve Ltd., Istanbul, Turkey). The remainder was centrifuged at 3000*g* for 10 min within 20 min of sampling. Plasma was separated, stored in plastic tubes, and refrigerated at 4 °C before testing.

For determination of whole blood BHBA and glucose concentrations, the Optium Xceed was operated according to the manufacturer's operating guidelines (Anonymous, 2006a), and quality control reagents (Anonymous, 2006b) were run on a daily basis before any samples were analysed. The device measures glucose and BHBA in whole blood by enzyme-based electrochemical technique. Briefly, when the blood sample is applied to the glucose or β-ketone test strip, the glucose or BHBA in the blood react with the chemical in the test strip producing a small electrical current. The current is measured and the sensor then displays a result. The size of the current depends on the amount of glucose or BHBA in the blood sample (Anonymous, 2006a). According to the manufacturer's reports, the version of the hand-held glucose/ketone meter system used in this study performs blood glucose and BHBA measurements, with test times of 20 and 10 s, and sample volumes of 0.6 and 1.5 μL, respectively. The analytic measurement range is given as 1.1–27.8 mmol/L for glucose and 0–8.0 mmol/L for BHBA (Anonymous, 2005, 2006c).

After cowside blood BHBA and glucose testing with the hand-held meter, plasma concentrations of these parameters were analysed on the same day using commercially available reagents in the laboratory. A chemistry analyser (Merck Microlab 200, Darmstadt, Germany) was used to measure the plasma BHBA and glucose concentrations by D-3-hydroxybutyrate dehydrogenase (Randox Laboratories Ltd., Co. Antrim, UK) and hexokinase (Diasis Ltd., Istanbul, Turkey) assays according to the instructions of the test kit manufacturer, respectively. These enzymatic measurements were used as the reference laboratory methods or expressed as the comparison methods. One researcher (Voyvoda) performed all measurements.

Statistical analyses were performed in Excel spreadsheet (Microsoft Corporation, Redmond, WA, USA) using Analyse-it software (Analyse-it Software Ltd., Leeds, UK). Previously, the data were checked for normality using the Kolmogorov–Smirnov test. Both BHBA and glucose concentrations were normally distributed; therefore, the data from each parameter were expressed as mean ± standard deviation (SD). The significance of difference between means was evaluated by Student's *t*-test for paired samples. Blood glucose concentrations between subclinical ketotic cows (BHBA $\geqslant$ 1200 or 1400 μmol/L) and nonketotic cows (BHBA < 1200 μmol/L) were compared by using the Student's unpaired *t*-test. The correlation of results obtained by the two methods was assessed by performing the Pearson correlation. Agreement between methods was verified using the Bland–Altman difference plot (Bland and Altman, 1986) and the Passing–Bablok regression (Passing and Bablok, 1983) using the laboratory methods as the comparison methods (Jensen and Kjelgaard-Hansen, 2006). The differences were considered significant at values of $p < 0.05$.

The hand-held meter ketone test was evaluated using plasma concentrations of BHBA as the gold standard for the determination of SCK. Cows with BHBA concentration $\leqslant$ 3000 and $\geqslant$1200 μmol/L (Geishauser et al., 1998; Stöber, 2002; Sakha et al., 2007) or $\geqslant$1400 μmol/L (Duffield, 2000; Geishauser et al., 2000, 2001; Carrier et al., 2004; Oetzel, 2004) were considered to suffer from SCK, and denoted as true positive whereas plasma BHBA concentration lower than 1200 μmol/L was defined as nonketotic. With regard to the positive SCK criteria defined, the sensitivity, specificity, positive predictive value (p*V*+; proportion of cows that tested positive that were subclinically ketotic), and negative predictive value (p*V*−; proportion of cows that tested negative that were not ketotic) were calculated to detect the validity of hand-held meter BHBA testing for SCK.

3. Results

For this study, SCK was defined as plasma BHBA concentrations $\geqslant$1200 or $\geqslant$1400 μmol/L. Using plasma BHBA $\geqslant$ 1200 μmol/L as

the threshold value, 33.3% (26/78) of tested cows were considered as subclinically ketotic, with the proportion within herd ranging from 14.3% to 42.1%. Cows with SCK were detected in all the investigated dairies. Raising the threshold of plasma BHBA to 1400 μmol/L and higher, twenty cows (25.6%) were diagnosed as having SCK (Table 1). Of the 78 cows studied, 32.0% (25/78) and 24.3% (19/78) had SCK with ⩾1200 and 1400 μmol of BHBA/L of EDTA–anticoagulated whole blood measured by using the hand-held device, respectively. Cows with SCK were 4–8 years old with 2–6 lactations. The majority of SCK occurred in the first three weeks of lactation, with few cases thereafter.

The means ± SD of measured BHBA and glucose concentrations using two different methods are showed in Table 2. BHBA concentrations varied from 300 to 2900 μmol/L with the hand-held device and from 200 to 3000 μmol/L with the laboratory method. Overall, the BHBA results of the two methods were significantly different (Student's *t*-paired test, $p < 0.01$), with hand-held meter results in whole blood containing EDTA higher than laboratory results in plasma by a mean difference of 36.7 μmol/L (Table 2). However, direct comparison of BHBA data from the hand-held meter and the laboratory method revealed that there was excellent correlation between the two methods ($r = 0.97$). The Bland–Altman difference plot in Fig. 1A is shown with the bias between the two methods by plotting for each sample the difference of results from the two methods (*y*-axis) compared with the mean of results (*x*-axis). Examination of the difference plot exposed a slight positive bias of 36.7 μmol/L with narrow confidence interval (CI) (95% CI: 6.7–66.8 μmol/L) and narrow limits of agreement. Overall, the central 0.95 interval (mean difference, ±2SD) indicated a good agreement between the methods for BHBA measurement (Fig. 1A). Only one outlier was observed, where the difference between the hand-held meter and laboratory values was of potential significant (1400 vs. 1090 μmol/L) (Fig. 1A). Passing–Bablok's regression analysis also showed a linear relationship between the two methods in the studied BHBA concentration range (Fig. 1B).

Table 1
Frequency table for cows categorized as having SCK based on plasma BHBA ⩾ 1200 or ⩾1400 μmol/L.

Dairy no.	Number of selected cows in each dairy	Number of cows with SCK[a]	
		BHBA ⩾ 1200 μmol/L	BHBA ⩾ 1400 μmol/L
1	21	3 (14.3%)	2 (9.5%)
2	19	8 (42.1%)	6 (31.6%)
3	8	3 (37.5%)	2 (25.0%)
4	17	7 (41.2%)	5 (29.4%)
5	13	5 (38.5%)	5 (38.5%)
Total	78	26 (33.3%)	20 (25.6%)

[a] Numbers in parenthesis indicate percentage of cows with SCK.

Table 2
Mean ± SD of BHBA and glucose concentrations measured using two different methods overall and cows with (BHBA ⩾ 1200 or ⩾1400 μmol/L) and without (BHBA < 1200 μmol/L) SCK.

Parameter	Method	
	Laboratory	Hand-held meter
BHBA (μmol/L)		
All cows	1077.4 ± 576.2 ($n = 78$)	1114.1 ± 570.0* ($n = 78$)
Cows with BHBA < 1200	724.2 ± 193.8 ($n = 52$)	798.1 ± 219.7 ($n = 53$)
Cows with BHBA ⩾ 1200	1748.1 ± 494.1 ($n = 26$)	1784.0 ± 499.7 ($n = 25$)
Cows with BHBA ⩾ 1400	1902.0 ± 460.9 ($n = 20$)	1947.4 ± 463.5 ($n = 19$)
Glucose (mmol/L)		
All cows	3.25 ± 0.42 ($n = 78$)	3.52 ± 0.48** ($n = 78$)
Glucose at BHBA < 1200 μmol/L	3.38 ± 0.35 ($n = 53$)	3.65 ± 0.45 ($n = 53$)
Glucose at BHBA ⩾ 1200 μmol/L	2.98 ± 0.42*** ($n = 26$)	3.25 ± 0.45*** ($n = 25$)
Glucose at BHBA ⩾ 1400 μmol/L	2.89 ± 0.40*** ($n = 20$)	3.14 ± 0.42*** ($n = 19$)

* Statistically different from the laboratory method $p < .01$.
** Statistically different from the laboratory method $p < .001$.
*** Statistically different from the glucose at BHBA < 1200 μmol/L, $p < .001$.

Glucose concentrations varied from 2.11 to 4.72 mmol/L with the hand-held device and from 1.78 to 4.05 mmol/L with the laboratory method. Overall, glucose concentrations were significantly ($p < 0.001$) higher with the hand-held meter than with the laboratory method (Table 2). The Pearson correlation analysis between glucose concentrations obtained with the two methods was fair but highly significant ($r = 0.63$, $p < 0.001$). Bland–Altman difference plot revealed a small, but real, positive bias with narrow CI (95% CI: 0.182–0.358); however, the limits of agreement were wide (Fig. 2A). The blood glucose concentrations measured with the hand-held meter may vary from 1.04 above to 0.50 mmol/L below the values obtained with the reference laboratory method (Fig. 2A). Outliers of potential significance were identified in four samples, where the hand-held meter values were higher than those of laboratory reference method (4.22 vs. 2.59 mmol/L, 4.50 vs. 3.21 mmol/L, 3.77 vs. 2.64 mmol/L, 4.77 vs. 3.61 mmol/L) (Fig. 2A). Passing–Bablok analysis revealed a nonlinear correlation between the two glucose measurement methods with a slope of 1.10 (95% CI: 0.95–1.34) and an *y*-intercept (−0.06) close to zero (Fig. 2B). From the Passing–Bablok regression and the Bland–Altman plot results, the agreement was not as good for blood glucose as it was for blood BHBA. Glucose concentrations in the subclinical ketotic cows were significantly ($p < 0.001$) lower than in the cows without SCK at two different threshold values for SCK in both methods (Table 2). Analysis of pooled data from all 78 cows revealed also that there was a significant negative correlation between BHBA and glucose measured with laboratory reference methods ($r = -0.64$; $p < 0.01$), while the correlation coefficient between BHBA and glucose measured with the hand-held meter was −0.54 and significant ($p < 0.01$).

The test performance of the hand-held meter relative to plasma BHBA ⩾ 1200 or ⩾1400 μmol/L for detection of SCK is shown in Table 3. At a threshold of ⩾1400 μmol/L, the hand-held meter had better combined sensitivity and specificity. A sensitivity of 90% and a specificity of 98% yielded p*V*+ and p*V*− of 95% and 97%, respectively (Table 3). From a specificity of 98%, the hand-held meter BHBA testing provides 2% false positives.

4. Discussion

In cattle practice, rapid and reliable tests are urgently needed for diagnosing or monitoring of diseases. Results of this study indicated that the hand-held meter without additional calibration or adjustment from the human system provides accurate and rapid BHBA measurement in whole blood and can be used as a cowside test for detecting SCK in individual cows as well as at the herd level.

Threshold values to separate healthy cows from cows with SCK have been reported in different studies to vary between approximately 1000 and 1400 μmol/L of BHBA in blood (Duffield, 2000; Geishauser et al., 2000; Stöber, 2002). However, serum BHBA concentration ⩾1200 μmol/L (Geishauser et al., 1998; Stöber, 2002; Sakha et al., 2007) or ⩾1400 μmol/L (Nielen et al., 1994; Geishauser et al., 2000; Duffield, 2003; Carrier et al., 2004; Oetzel, 2004; Rollin, 2006) was commonly assumed as a threshold value for SCK. The

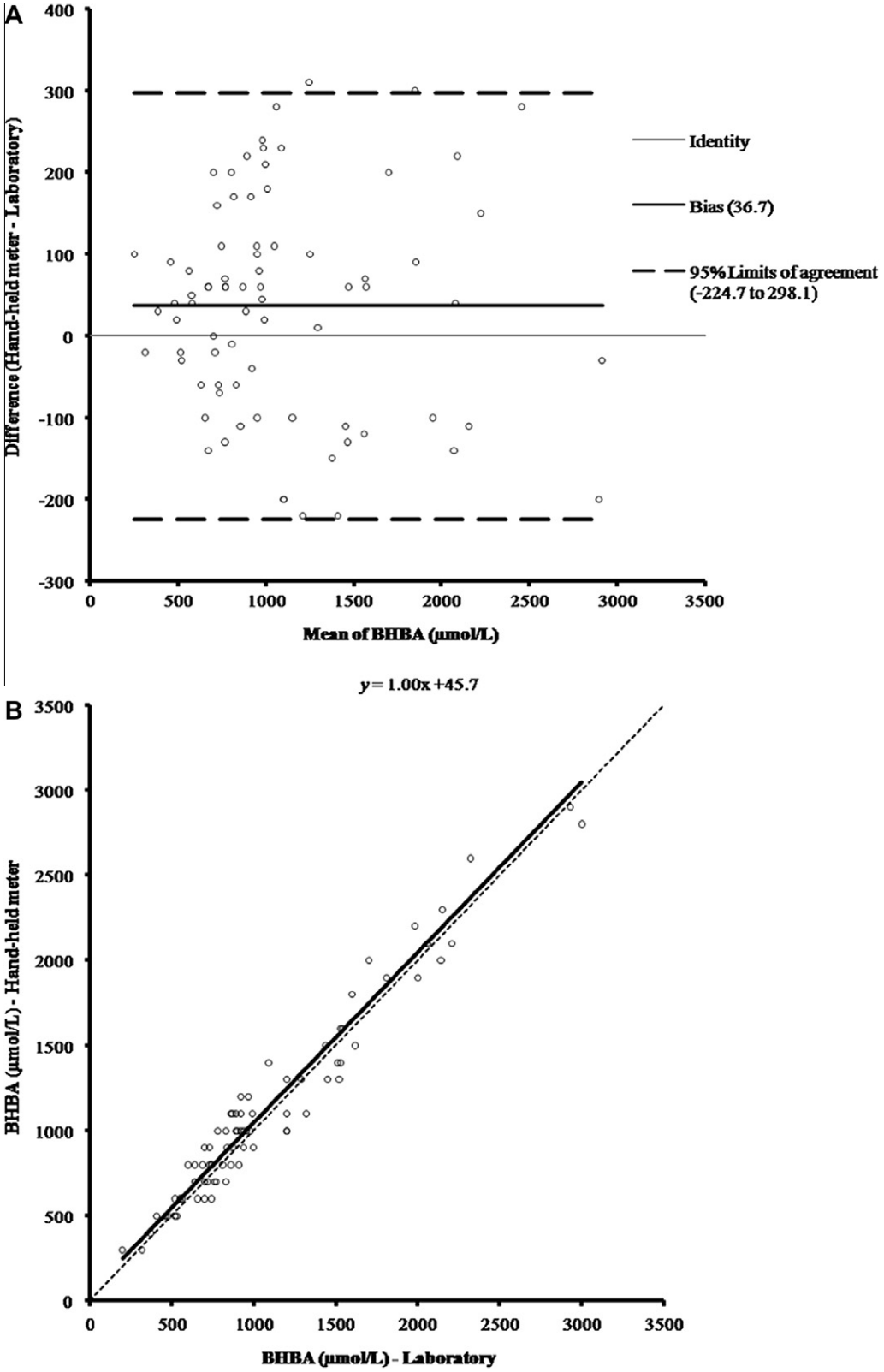

Fig. 1. Comparison between hand-held meter and laboratory methods for measurement of BHBA in blood samples from 78 cows. (A) Bland–Altman's difference plot. (B) Scatter plot with Passing–Bablak regression. The dotted line represents equivalence; the solid line indicates Passing–Bablak regression (with 95% confidence intervals in parenthesis): $BHBA_{hand\text{-}held\ meter} = 1.00(0.94/1.08) \times BHBA_{laboratory} + 47.50(-28.38/99.41)$.

exact threshold chosen usually has a minor effect on the interpretation of herd-based results (Oetzel, 2004). Clinical ketosis generally involves much higher levels ($\geqslant$3000 µmol/L) of blood BHBA (Stöber, 2002; Oetzel, 2004). In this study, blood BHBA concentrations from 78 cows ranged from 200 to 3000 µmol/L, and a threshold of 1200 µmol/L was exceeded in the laboratory method (33.3%) and the hand-held meter (32.0%). Testing with the two methods at a threshold of 1400 µmol/L resulted also in similar percentage of cows

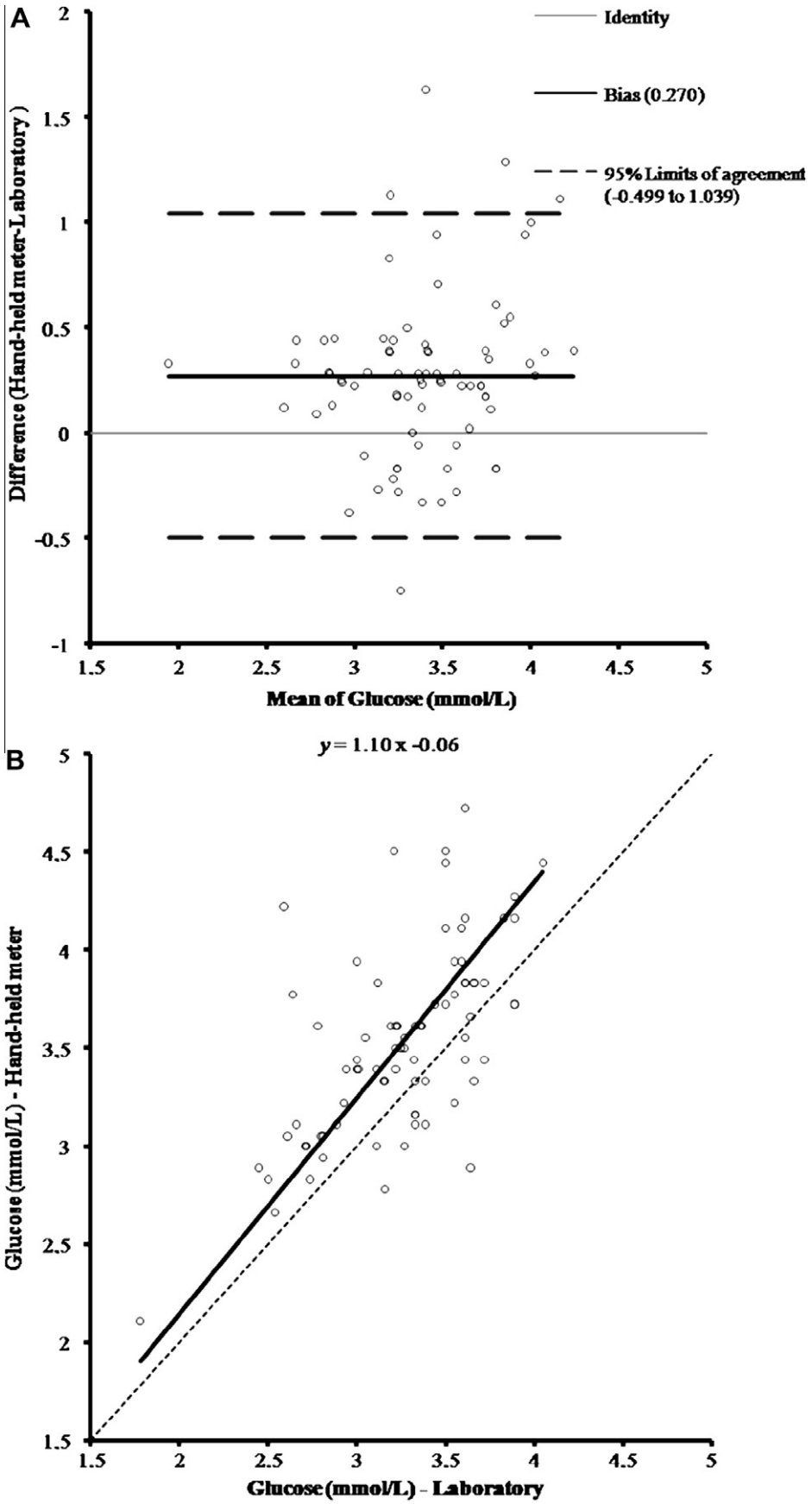

Fig. 2. Comparison between hand-held meter and laboratory methods for measurement of glucose in blood samples from 78 cows. (A) Bland–Altman's difference plot. (B) Scatter plot with Passing–Bablak regression. The dotted line represents equivalence; the solid line indicates Passing–Bablak regression (with 95% confidence intervals in parenthesis): $\text{glucose}_{\text{hand-held meter}} = 1.00(0.95/1.34) \times \text{glucose}_{\text{laboratory}} - 0.06(-0.87/0.42)$.

Table 3
Performance of the hand-held meter for diagnosing SCK in cows at two different threshold values of plasma BHBA.

Threshold values	Sensitivity (%)	Specificity (%)	pV+ (%)	pV− (%)
BHBA ⩾ 1200 μmol/L	85	94	88	91
BHBA ⩾ 1400 μmol/L	90	98	95	97

with SCK (25.6% vs. 24.6%). The diurnal variation in BHBA concentrations, which is related to feeding (Eicher et al., 1998; Duffield, 2000), was not considered a problem in our study, because we sampled each cow for blood at fixed times. Therefore, post feeding high levels of BHBA would be found in blood with the laboratory method and the hand-held meter, causing no false conclusions for the test characteristics. The proportions of cows with SCK cannot be considered as representative because of the limited number of cows used; however, the disease seems to an important problem in dairy herds of Aydin province, Turkey.

To measure blood BHBA concentrations in this study, the hand-held meter gave significantly higher values in comparison with the laboratory methods (Table 2), although there was an excellent correlation ($r = 0.97$) and a good agreement between the two BHBA measurements (Fig. 1A and B). The higher BHBA values for the hand-held meter in this could be explained by using blood containing EDTA because our results were different from the results by Iwersen et al. (2009) who used whole blood without anticoagulant and found slightly lower BHBA values on the hand-held meter compared to the laboratory standard. A significant difference between mean values from two methods can be clinically irrelevant. The significant difference between BHBA values in this study depended mainly on the paired test used for group comparisons, which emphasizes the presence of a general trend to increased (or decreased) values between the two groups rather than reflecting a true difference in mean values (Bland and Altman, 1986). Moreover, two different methods can show a perfect correlation coefficient ($r = 1$) but have a significant bias between them (Bland and Altman, 1986; Jensen and Kjelgaard-Hansen, 2006). From a clinical and biological point of view, BHBA measured with the hand-held meter or the laboratory method can be used interchangeably because the BHBA results from the hand-held meter correlated excellently and aggregated well with laboratory results taken as gold standard (Fig. 1A and B). In fact, the hand-held meter overestimated BHBA concentrations (bias = +36.7 or expressed as a percentage, 4.4%); however, only one outlier was recorded, where the difference between the hand-held meter and laboratory values was of potential significant (1400 vs. 1090 μmol/L) (Fig. 1A). Our result confirms findings in few previous reports in cattle (Kupczyński and Cupok, 2007; Oetzel and McGuirk, 2007; Iwersen et al., 2009) and dogs and cats (Hoenig et al., 2008) as well as several human medicine studies (Byrne et al., 2000; Chiu et al., 2002; Anonymous, 2006c; Noyes et al., 2007; Ronald, 2008; Yoong et al., 2008) showing that BHBA measurement made using the hand-held meter correlated well with the laboratory methods. The first report about the application of this hand-held meter to cattle was an abstract in year 2004 in which no accuracy information was included (Endecott et al., 2004). More recent studies demonstrated that BHBA results from the hand-held meter were highly correlated to laboratory test results, with a correlation coefficient varying from 0.92 to 0.99 (Jeppesen et al., 2006; Kupczyński and Cupok, 2007; Oetzel and McGuirk, 2007; Iwersen et al., 2009), and there was a good agreement between two measurements (Kupczyński and Cupok, 2007; Oetzel and McGuirk, 2007). The excellent correlation ($r = 0.97$) and the good agreement (Fig. 1A and B) between the two BHBA measurements in the present study support the results of the above-mentioned studies. As described (Oetzel and McGuirk, 2007), those results may be attributed to the analytical principle that is same in both techniques.

The main interest of hand-held glucometers in cattle is found in neonatology and every time a glucose-containing solution is infused intravenously (Rollin, 2006). Furthermore, they could be helpful for diagnosing and differentiating type I ketosis (Herdt, 2000; Rollin, 2006), although measurement of glucose concentration is not a very good indicator of the energy status of dairy cows (Rollin, 2006). Similar to BHBA results, glucose results of the hand-held meter were significantly higher than those obtained by the laboratory method (Table 2). This result was comparable with results by Kupczyński and Cupok (2007) which used EDTA–anticoagulated whole blood and found slightly higher glucose values on the hand-held meter compared to the laboratory method in dairy cows. In contrast to BHBA results, there was a fair correlation ($r = 0.63$) between the two glucose measurement methods. Our result differed slightly (fair correlation: $r = 0.59$–0.79) from the results of few recent studies which gave a correlation of or about 0.78 (Kupczyński and Cupok, 2007; Oetzel and McGuirk, 2007). Correlation coefficient measures the strength of a relation between two variables, not the agreement between them (Bland and Altman, 1986). Therefore, the fair correlation between the glucose values from the two methods in this study may not be clinically significant. Passing–Bablok regression and Bland–Altman plot, more informative analysis than a regression analysis (Bland and Altman, 1986), for method comparison indicated that the agreement was not as good for blood glucose as it was for blood BHBA (Figs. 1 and 2). Interpretation of the bias data obtained by the Bland–Altman plot in isolation could be misleading, and limits of agreement may be more appropriate (Bland and Altman, 1986; Jensen and Kjelgaard-Hansen, 2006). From the results of the Bland–Altman plot in this study, 95% limits of agreement were so wide that the blood glucose concentrations measured with the hand-held meter may vary from 1.04 mmol/L (18.7 mg/dl) above to 0.50 mmol/L (9.0 mg/dl) below the values obtained with the reference laboratory method (Fig. 2A). Thus, blood glucose measurement made using the hand-held meter may lead particularly at low glucose concentrations to misinterpretation of the results in dairy cows. Our result was similar to reports by Rollin (2006) and Oetzel and McGuirk (2007) who denoted less accuracy of the hand-held portable glucometers in generally and the Optium Xceed for measuring blood glucose concentrations in dairy cows. On the other hand, our findings differ from glucose results obtained on human whole blood with the hand-held meter (Batki et al., 2002; Solnica et al., 2003; Anonymous, 2005; Ronald, 2008) and on cattle whole blood with other glucometers (Roeder et al., 1996; Rumsey et al., 1999), when compared with those using laboratory reference method in plasma gave a strong correlation and a good agreement. The reason for the different results of this study with others is not evident. It is well known that the same method can show a significant variability under various conditions. The operating range of the hand-held meter is given as 10–50 °C for ambient temperature and 10–90% for relative humidity (Anonymous, 2006a). According to the test strip instruction for humans, best results can be obtained when blood glucose strips are used at temperatures between 15 and 40 °C (Anonymous, 2005). Also, hematocrit values can significantly affect glucose measurements in human blood determined using hand-held glucose meters. In general, a high hematocrit was associated with underestimation, while a low hematocrit with overestimation of glucose results (Tang et al., 2000). The manufacturer states that the results are not affected by hematocrit in the range of 20–70% at a glucose level of ⩽16.7 mmol/L (Anonymous, 2005). Hematocrit values for 78 cows involved in this study varied from 32% to 43%. Since the device during this study period has been used within acceptable weather conditions, and hematocrit values of animals used were in the testing limits as recommended by the manufacturer, these factors influencing the accuracy of glucose analysis can be ex-

cluded. Both methods used were based on an enzymatic reaction; therefore, methodical variability also can be excused. However, fair agreement may be due to the measurement technique because the hand-held meter has been developed and standardized for monitoring human blood. Hand-held glucose meters in general measure glucose in the red blood cells (RBCs) and the plasma, and the glucose from the RBCs equilibrates with the glucose from the plasma portion as the test is being performed (Batki et al., 2002; Solnica et al., 2003). Nevertheless, the distribution of glucose between RBCs and plasma in whole blood samples is different between humans (Mackay, 1932) and some other mammals (Coldman and Good, 1967). In a study by Cozzi et al. (2006), two hand-held glucose meters designed for human use produced results in dogs that differed by as much as 39% compared to the reference laboratory results whereas a hand-held glucose monitoring device specifically developed for veterinary use provided glucose results that were statistically equivalent (2%) to the reference laboratory results.

A significant difference between glucose concentrations of cows with and without SCK (Table 2) and a fair but significant negative correlation between BHBA and glucose concentrations observed in this study are similar to the results by Sakha et al. (2007) and in accordance with the fact that hypoglycaemia is the driving force in bovine subclinical and clinical ketosis that ultimately causes ketonaemia (Bruss, 1997; Stöber, 2002). However, in the disease, dairy cattle can become ketonaemic without the presence of significant hypoglycaemia (Oetzel, 2004). Consequently, it was not surprising that blood glucose was not strongly correlated with BHBA concentration of blood in this study.

The hand-held meter BHBA testing showed a sensitivity of 85% and a specificity of 90% compared with plasma BHBA levels of 1200 μmol/L and higher. Using 1400 μmol/L of plasma BHBA for the definition of SCK, its sensitivity and specificity were 90% and 98%, respectively (Table 3). At a threshold of 1400 μmol of BHBA/L of blood, its good specificity would assure few (2%) false positives. These results also aggressed with few current studies (Kupczyński and Cupok, 2007; Oetzel and McGuirk, 2007; Iwersen et al., 2009) in which the sensitivity and specificity of the hand-held meter range from 85% to 100%, and from 97% to 100%, respectively. A great variety of cowside urine and milk tests are available for SCK monitoring of dairy herds but none of these have perfect sensitivity and specificity in comparison with blood BHBA (Oetzel, 2004; Iwersen et al., 2009). Urine ketone tests in general have good sensitivity but poor specificity (Oetzel, 2004; Rollin, 2006). This makes them a useful test for evaluating individual sick cows but not for herd monitoring with regard to SCK. Ketone cowside milk tests based on nitroprusside reaction are generally not as sensitive as urine tests (Oetzel, 2004; Rollin, 2006). Oetzel (2004) summarized performance of a semi-quantitative test strip measuring BHBA in milk relative to serum BHBA $\geqslant$ 1400 μmol/L from published studies and noted that the sensitivity and specificity of the test for SCK are fairly dependent on threshold values used which influence individual and herd-based usefulness of the test for diagnosing or monitoring of SCK. Using a threshold of BHBA $\geqslant$ 1400 μmol/L in plasma that is mostly recommended to distinguish between cows with and without SCK (Nielen et al., 1994; Geishauser et al., 2001; Duffield, 2003; Carrier et al., 2004; Oetzel, 2004; Rollin, 2006), the hand-held meter used in this study provided a sensitivity of 90%, and a specificity of 98% (Table 3). It has much better combined sensitivity and specificity than those of currently available urine and milk cowside tests summarized by Oetzel (2004), and can be used as cowside blood test for detecting SCK in individual cows as well as at the level of the herd.

Several tests can be utilized by the practitioner in his daily routine to effectively confirm diagnosis at the herd or animal level. Ideally, test procedures in cattle practice should provide rapid and reliable results, require minimal and robust instrumentation and be inexpensive. The hand-held meter used in this study meets all these conditions. The last version of the hand-held meter produces accurate BHBA results in 10 s per sample, requires 1.5 μL of whole blood and has few operator dependent steps. An easy blood measurement of BHBA concentration could diagnose SCK earlier than cowside urine and milk testing routinely used because BHBA and other ketones have to reach a transport maximum before they are excreted in urine and milk (Bruss, 1997; Laffel, 1999). Moreover its cost is affordable, as much at the time of acquisition of the apparatus (about $70 USD) as when functioning (about $4/BHBA test). The cost per BHBA test is normally cheaper than sending a blood sample to a laboratory for BHBA testing.

In conclusion, the results of this study showed that the hand-held meter is rapid and valid for measuring blood BHBA concentrations and can be used as a cowside test for detection of SCK whereas it is insufficiently accurate for use in clinical practice to determine blood glucose concentrations in dairy cows.

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