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Accuracy of two hand-held electronic devices for determination of blood β-hydroxybutyrate in dairy cows

Acurácia de dois testes eletrônicos portáteis para dosagem de β-hidroxibutirato sanguíneo de vacas leiteiras

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SUMMARY

The cowside determination of blood β-hydroxybutyrate (BHB) is an important tool for diagnosing subclinical ketosis in dairy cattle. Portable methods to measure BHB have been introduced in the past years. This study evaluated the accuracy of two hand-held electronic devices for blood determination of BHB in dairy cows at early postpartum. A total of 98 blood samples were collected from dairy cows in the first month of lactation and tested with FreeStyle Optium (FSO, Abbott) and KetoVet (KVE, TaiDoc) portable devices according to the manufacturer instructions. Spectrophotometric BHB analysis (Ranbut, Randox) was used as standard method. The incidence of subclinical ketosis was 37.7 % determined by the standard method, 40.8 % determined by the FSO system and 42.8 % detected with the KVE system. The sensitivity and specificity indexes were 88.1% and 98.4% for FSO and 78.7% and 92.4% for KVE, respectively. The Pearson´s correlation coefficients comparing the portable devices to the standard technique were 0.96 for FSO and 0.93 for KVE. No significant difference in BHB values was found between the two portable tests and the standard method. Predictive values (PV) were better using FSO (positive PV 97.3 %, negative PV 92.4%) than using KVE (positive PV 88.1 %, negative PV 85.9 %). Passing-Bablok regressions revealed good agreement between methods. Though FSO system had a better performance than KVE system, the results suggest that the two portable systems have good accuracy and are reliable for measuring BHB.

Keywords: ketosis, predictive value, sensitivity, specificity

RESUMO

A dosagem de β-hidroxibutirato (BHB) sanguíneo é uma importante ferramenta para diagnosticar cetose subclínica em vacas leiteiras. Nos últimos anos vários dispositivos portáteis para medir BHB têm sido introduzidos no mercado. Este estudo objetivou avaliar a acurácia de dois aparelhos eletrônicos portáteis para dosagem sanguínea de BHB de vacas leiteiras no início do pós-parto. Um total de 98 amostras de sangue de vacas leiteiras foram testadas durante o primeiro mês de lactação nos dispositivos FreeStyle Optium (FSO, Abbott) e KetoVet (KVE, TaiDoc) de acordo às instruções do fabricante. Como padrão-ouro foi feita dosagem espectrofotométrica de BHB (Ranbut, Randox). A incidência de cetose subclínica foi de 37,7 % usando a técnica do padrão-ouro, 40,8% usando o sistema FSO e de 42,8 % usando o sistema KVE. Os índices de sensibilidade e especificidade foram de 88,1 % e 98,4 % para FSO e 78,7 % e 92,4 % para o KVE, respectivamente. Os coeficientes de correlação de Pearson comparando os aparelhos portáteis com a técnica do padrão-ouro foram de 0.96 para FSO e de 0.93 para KVE. Não houve diferença significativa nos valores médios de BHB entre os dois testes com o padrão-ouro. Os valores preditivos (VP) foram melhores para FSO (VP positivo 97.3 %, VP negativo 92.4%) do que para KVE (VP positivo 88.1 %, VP negativo N 85.9 %). Desta forma, embora FSO tivesse um melhor desempenho sobre KVE, sugere-se que os dois sistemas portáteis estudados podem ser usados na mensuração de BHB com acurácia e confiabilidade.

Palavras-chave: cetose, especificidade, sensibilidade, valor preditivo

INTRODUCTION

The main challenge faced by cows during early postpartum is the significant increase in nutrients demand for milk production associated with low intake of dry matter causing large mobilization of body reserves for energy needs (HERDT, 2000). The serum concentration of non-esterified fatty acids (NEFA) increases to serve as a source of energy since glucose is being directed to the lactogenic and fetal development (ALLEN & PIANTONI, 2013). However, when the amount of triglycerides that reaches the liver exceed the capacity of oxidation, partial oxidation of NEFA occurs resulting in increased production of ketone bodies, mainly beta-hydroxybutyrate (BHB).

High concentrations of blood BHB cause economic losses to the dairy industry because it decreases milk production and reproductive efficiency (WALSH et al., 2007), and generates costs of treatment and increased culling of animals. Cows affected by ketosis were 1.8 and 1.6 times more likely to develop metritis and ovarian cysts, respectively, than healthy cows (GRÖHN et al., 2003).

Monitoring of high-yielding dairy cows for ketosis in the postpartum period is considered beneficial and strongly recommended (OETZEL, 2004). Ketosis has been classified as clinical and subclinical. Clinical presentation is characterized by highly increased ketone bodies in blood, along with visible clinical signs, such as loss of appetite, rapid weight loss and hard stools (GORDON et al., 2013). Subclinical form is defined as the pre-clinical stage of ketosis, characterized by increase of blood concentration of ketone bodies (DUFFIELD et al., 2009) with blood BHB level above 1.2 mmol/L (IWERSEN et al., 2009).

Measurement of BHB in blood or serum is the only efficient method for detecting subclinical ketosis (VANHOLDER et al., 2015). Some cowside ketone tests have limitations in their use because they detect only acetoacetate and acetone through the nitroprusside reaction (Rothera test) but not BHB (GEISHAUSER et al., 1998) which is the predominant ketone body. Thus, they do not perform an accurate assessment of subclinical ketosis. In recent years, various portable electronic devices, originally developed for the human medicine, have been used for rapid determination of BHB concentration in dairy herds in clear advantage over the traditional cowside tests.

Therefore, the objective of this study was to evaluate the accuracy of two portable electronic testing available in the international market for the BHB measurement in blood of dairy cattle.

MATERIALS AND METHODS

All procedures with the cows of this study were approved by the Ethics Committee for Animal Use of the Federal University of Rio Grande do Sul and authorized by the herd owners (Project nº 27953). The experiment was conducted in two dairy herds located in the western region of the Santa Catarina state (southern Brazil). One herd had 500 Holstein cows confined in a free-stall system, and the other herd has 200 Holstein cows in semi-confined system. Ninety-eight blood samples (55 cows in free-stall system and 35 cows in semi-confined system) were collected from cows according to their period of lactation at the time of collecting

samples. All sampled animals were between the first and the fourth week of lactation. The diet of the animals was composed of corn silage, pastures (ryegrass, oats and barley) plus hay and concentrate. The concentrate contained corn, soybean meal and mineral supplement.

Two portable electronic devices to determine BHB in whole blood were used, consisting of a portable meter and a test strip: FreeStyle Optium β-ketone (Abbott Diabetes Care, UK) (FSO) and Ketovet (TaiDoc Technology Corporation, Taiwan) (KVE). The first device is used in diabetic humans, dogs and cats for measuring both glucose and BHB (VOYVODA & ERDOGAN, 2010) and the second one is a recently available electronic device with intended use specifically in cows. The KVE system requires a calibration strip with known value, provided with the device.

Blood samples were collected before the morning milking session from coccygeal vein puncture with a 21-G needle using plain vacuum tubes (BD Vacutainer, Becton Dickinson, USA). Immediately after collection, the blood was tested simultaneously on both devices. A drop of whole blood was disposed in the sample application zone of the strip, initiating an identical chemical reaction on the two devices as follows: the BHB in the sample is oxidized to acetoacetate by BHB dehydrogenase using the coenzyme NAD^+ that is reduced to NADH. Then, NADH is reoxidized to NAD^+ by a mediator, releasing electrons and generating an electric current which is directly proportional to the concentration of BHB (IWERSEN et al., 2013). The result is displayed on the screen in 5 seconds (KVE) or 10 seconds (FSO) after the application of the sample.

After clot formation in the tube (approximately 1 hour), the sample was centrifuged at 2,200 rpm for 10 minutes. The serum obtained was stored in two aliquots of 2 mL in plastic sterile microtubes tubes and stored at -18°C for a maximum time of three weeks until the measurement of BHB by a colorimetric enzymatic reaction (Ranbut, Randox Laboratories, UK) with an automatic biochemical analyzer (Wiener Lab CM 200, Argentina). The method is also based on the oxidation of BHB to acetoacetate by the enzyme BHB dehydrogenase and production of the reduced coenzyme NADH, which UV absorbance change is detected being proportionally to BHB concentration in the sample (MCMURRAY et al., 1984). This value of BHB determined at the laboratory was used as the standard method.

Statistical analysis was performed using the MedCalc program (MedCalc Software, Belgium). Normality of the data was checked through the Kolmogorov-Smirnov test. Mean and standard deviation was obtained for each test, and a Student´s *t*-test for paired samples was used for comparing means of the portable systems with the standard method. Significant level was established at $P< 0.05$. Pearson´s correlation coefficients between each test and the standard method were obtained, and sensitivity and specificity indexes were calculated for diagnosing subclinical ketosis, using a cut-off point of 1.2 mmol/L (IWERSEN et al., 2009). The sensitivity index was defined as the percentage of serum samples with BHB concentrations above 1.2 mmol/L correctly diagnosed as positive by the portable test according to the standard method. Specificity was defined as the percentage of samples with BHB concentrations below 1.2 mmol/L correctly diagnosed as negative by the portable test. Agreement between methods was checked through Passing-

Bablok regressions using the standard method (laboratory technique) as the comparison method. Positive predictive value (PPV), that is the probability of being positive when the result is positive, was calculated as follows: $PPV = \frac{TP}{TP+FP}$ where TP: true positive and FP: false positive. Negative predictive value (NPV), that is the probability of being negative when the result is negative, was calculated as follows: $NPV = \frac{TN}{TN+FN}$ where TN: true negative and FP: false negative.

RESULTS AND DISCUSSION

The parameters of accuracy obtained in this study are shown in Table 1. From all blood samples analyzed in the laboratory by the spectrophotometric technique (standard method) 37 cows (37.75 %) exceeded the cut-off point (>1.2 mmol/L). The incidence of subclinical ketosis using the FSO device was 40.81 % and using the KVE device was 42.85 %. This means that portable devices possibly overestimate the BHB values. Using whole blood in portable devices and serum in spectrophotometric analysis is accepted for comparison considering the highly significant correlation coefficient (r= 0.94) between the two types of samples obtained by other authors (IWERSEN et al., 2013).
The data of the BHB values obtained by the three methods were normally distributed according to the Kolmogorov-Smirnov test. No significant differences were seen among BHB concentrations using the Student´s *t* test, in a paired comparison of the two devices with the standard method (P values: Ranbut vs FSO= 0.64; Ranbut vs KVE= 0.48).

Table 1. Measured parameters for evaluating accuracy of whole blood BHB values using portable FreeStyle Optium and KetoVet systems and laboratory analysis of serum BHB measured by spectrophotometric technique (standard)

Parameter	Technique		
	Spectrophotometric	FreeStyle Optium	KetoVet
Number of samples	98	98	98
BHB mean ± SD (mmol/L)	1.12 ± 0.57	1.11 ± 0.68	1.14 ± 0.61
Confidence interval (95%)	0.97 - 1.24	1.0 - 1.23	1.0 - 1.26
Average difference*	-	-0.01	+0.02
Pearson´s correlation coefficient	-	0.96	0.93
Incidence of SK (%)	37.75	40.81	42.85
Sensitivity (%)	-	88.10	78.72
Specificity (%)	-	98.39	92.42
PPV (%)	-	97.36	88.1
NPV (%)	-	92.42	85.91
Passing-Bablok regression*	-	y= 1,16 x - 0,18	y= 1,08 x - 0,04

BHB= beta-hydroxybutyrate; SD= standard deviation; SK= subclinical ketosis; PPV= positive predictive value; NPP= negative predictive value.
* Compared to spectrophotometric technique.

The Pearson´s correlation coefficients between the whole blood BHB concentration measured with the portable electronic systems and the serum BHB concentration observed at the laboratory were 0.96 for the FSO and 0.93 for KVE. While the KVE test had an average difference of +0.02mmol/L from laboratory results, the FSO test had an average difference of -0.01 mmol/L.

Based on the standard method, the results of sensitivity were 88.1% for the FSO test and 78.7 % for the KVE test and the specificity were 98.4 % to 92.42 %, respectively. Those values are considered very good for hand-held meters used for measuring BHB which there have been reported sensitivities ranging from 85 % to 100 % and specificities ranging from 97% to 100% (OETZEL, 2004; OETZEL & MCGUIRK, 2007) specially when compared with other tests for detecting ketones in urine or milk using the nitroprusside reaction (ROLLIN, 2006).

To our knowledge, this is the first study comparing FreeStyle Optium and KetoVet systems for the measurement of BHB in dairy cattle. The correlation coefficient (r= 0.96) and the average difference (-0.01 mmol/L) between the standard method and the FSO were similar to those reported by Iwersen et al. (2013) using the FSO System who founded r= 0.94 and average difference of +0.04 mmol/L and those reported by Voyvoda and Erdogan (2010) using the FSO system who reported r= 0.97 and average difference of +0.03 mmol/L. Iwersen et al. (2009) evaluating the Precision Xtra meter system, which uses the same technique of the two devices used in our study, found a correlation coefficient of r= 0.95 and average difference of -0,03 mmol/L. While in the study of Iwersen et al. (2009), the Precision Xtra system underestimated the laboratory values of BHB, the work of Iwersen et al. (2013) and Voyvoda and Erdogan (2010) overestimated data of BHB using the Precision Xtra meter and FSO systems, respectively. In our study, the average difference obtained by FSO system (-0.01 mmol/L) was very close to the laboratory BHB values.

Compared with FSO, the KVE system had a slightly lower correlation coefficient (r= 0.94) and a light higher average difference with the laboratory analysis (+0.02 mmol/L). Besides, the FSO test showed better specificity (98.4%) than KVE (92.4%) and better sensitivity (88.1 %), than KVE (78.7 %). Iwersen et al. (2009) found sensitivities between 88 and 96 % and specificities between 96 and 97%, at 1.2 and 1.4 mmol/L of blood BHB, respectively using the Precision Xtra device. The results obtained by those authors are more similar to the results obtained in our study with FSO system.

The Passing-Bablok regressions revealed good agreement between methods (Figure 1) without significant deviation from linearity (P=0.67), which was similar to the results obtained by Voyvoda and Erdogan (2010) measuring BHB with the FSO system.

Predictive values, which indicate the probabilities of considering positive a cow with subclinical ketosis when the result is positive (PPV), or considering negative when the result is negative (NPV), had better performances with FSO than with KVE (Table 1).

In dairy herd practice, portable and reliable tests are very important for diagnosing or monitoring subclinical and clinical ketosis. The problem with subclinical ketosis is that is a condition that can pass undetected and untreated with adverse effects on productivity (GARRO et al., 2014).

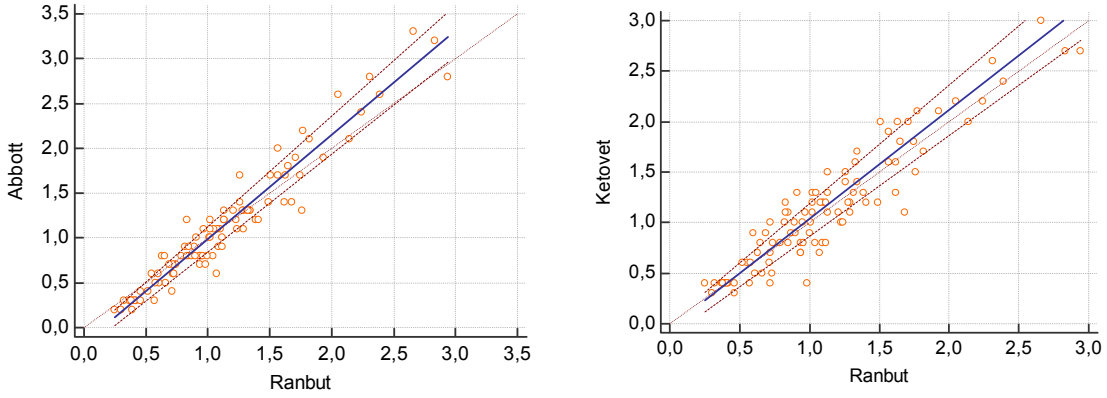

Figure 1. Scatter plot with Passing-Bablak regression for FSO (left) and KVE (right) compared to the standard method (Ranbut). All units are expressed in mmol/L. The test revealed no significant deviation from linearity. Regression equations are shown in Table 1

An opportune observation of a pre-clinical situation of ketosis through the BHB measurement will prevent economic losses due to veterinary treatment and milk yield drop. The results of the present study suggest that the hand-held meters evaluated provide accurate and rapid BHB measurement in whole blood.

Though FreeStyle Optium system had better agreement to the standard method than Ketovet system, from a clinical point of view, both hand-held meters evaluated in this study can be used interchangeably for measuring BHB because they correlated very well to the standard laboratory technique.

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