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Comparison of 2 electronic cowside tests to detect subclinical ketosis in dairy cows and the influence of the temperature and type of blood sample on the test results

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ABSTRACT

The objective of this study was to determine the suitability of 2 electronic hand-held devices [FreeStyle Precision (FSP), Abbott GmbH & Co. KG, Wiesbaden, Germany and GlucoMen LX Plus (GLX), A. Menarini GmbH, Vienna, Austria] for measuring β-hydroxybutyrate (BHBA) in dairy cows. Three experiments were conducted to evaluate (1) the diagnostic performance of the devices, (2) the effect of the type of blood sample, and (3) the influence of the ambient temperature on the determined results. A total of 415 blood samples from lactating Holstein and Simmental cows were collected and analyzed with both devices (whole blood) and in a laboratory (serum). Correlation coefficients between whole-blood and serum BHBA concentrations were highly significant, with 94% for the FSP and 80% for the GLX device. Based on thresholds for subclinical ketosis of 1.2 and 1.4 mmol of BHBA/L, results obtained with the hand-held devices were evaluated by receiver operating characteristics analyses. This resulted in adjusted thresholds of 1.2 and 1.4 mmol/L for the FSP and 1.1 and 1.3 mmol/L for the GLX device. Applying these thresholds, sensitivities were 98 and 100% for the FSP and 80 and 86% for the GLX device, respectively. Corresponding specificities were 90 and 97% for the FSP and 87 and 96% for the GLX device, respectively. Additionally, concentrations of BHBA were tested with both devices in whole blood, EDTA-added whole blood, and in their resulting serum and plasma, collected from 65 animals. Determined BHBA concentrations were similar within each device for whole and EDTA-added blood, and in serum and plasma, but differed between whole blood and serum and between EDTA-added blood and plasma. Blood samples with low (0.4 mmol/L), medium (1.1 mmol/L), and high (1.6 mmol/L) BHBA concentrations were stored between +5 to +32°C and analyzed repeatedly at temperature levels differing by 4°C. Additionally, devices and test strips were stored at equal conditions and used for measurement procedures. Storage temperature of the devices and test strips did not influence the differences between the results of the laboratory and the devices, whereas the temperature of the blood samples caused significant differences. Although the level of agreement between the laboratory and the GLX device was lower than for the laboratory and the FSP device, both devices are useful tools for monitoring subclinical ketosis in dairy cows. Due to their effects on the determined results, the type and temperature of the tested sample should be considered.

Key words: cow, ketosis, β-hydroxybutyrate, diagnostic test

INTRODUCTION

After parturition, the majority of all cows undergo a distinct period of negative energy balance. The reduction in feed intake shortly before parturition (Hayirli et al., 2002; Grummer et al., 2004) as well as the lagged increase in DMI within the first weeks of lactation causes a discrepancy between the energy required and consumed for lactation (Grummer, 2008). Some degree of negative energy balance and hence body fat mobilization with increased levels of NEFA is expected in the transition period (Ospina et al., 2010) and is a normal homeorhetic adaption of the cow with the onset of lactation (Bauman and Currie, 1980; Bell, 1995; Duffield et al., 2009). Excessive body fat mobilization, exceeding the liver's capacity to completely oxidize NEFA, is associated with high concentrations of ketones, as BHBA, acetone, and acetoacetate (**AcAc**). These excessive levels are indicative of maladaptation of the energy metabolism during early lactation (Suriyasathaporn et al., 1999; Duffield et al., 2009; LeBlanc, 2010).

Elevated concentrations of BHBA in blood are associated with an increased risk for periparturient metabolic disorders or infectious diseases (Cook et al., 2006a;

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Dubuc et al., 2010; Suthar et al., 2013). Furthermore, negative associations of elevated BHBA concentrations on milk production (Duffield et al., 2009; Chapinal et al., 2012) and reproductive performance (Walsh et al., 2007a; Chapinal et al., 2012) have been reported. Subsequently, affected cows are at higher risk to be removed from the herd within early lactation (Oetzel, 2004).

In the periparturient period, up to 50% of the animals suffer from metabolic or infectious diseases and approximately 75% of diseases in dairy cows occur within this time frame (LeBlanc, 2010). Therefore, monitoring high-producing dairy herds for subclinical diseases in the periparturient period (e.g., for ketosis and hypocalcaemia) is considered to be beneficial and is recommended by several authors (Geishauser et al., 1998; Oetzel, 2004; Cook et al., 2006a,b; LeBlanc, 2010).

Subclinical ketosis (**SCK**) within the first 2 wk of lactation has been defined as serum BHBA concentrations exceeding 1.2 and 1.4 mmol/L in serum (Duffield et al., 1998; Cook et al., 2006a; Duffield et al., 2009) without any clinical signs of the disease. The gold standard test for detecting SCK is to determine the concentration of BHBA in serum or plasma (Duffield et al., 1998), because of its stable characteristics in blood, compared with acetone and AcAc (Herdt, 2000). Various methods have been described for cowside BHBA testing in urine, milk and blood, with different test characteristics (Geishauser et al., 2000; Carrier et al., 2004; Iwersen et al., 2009). Recent studies based on blood BHBA concentrations reported incidences of SCK from d 3 to 16 of lactation between 26.4% and 55.7% in 4 North American dairy farms (McArt et al., 2012). Suthar et al. (2013) presented an overall prevalence of SCK of 21.8% (11.2 to 36.6%) for 10 European countries within 2 to 15 d after parturition using a threshold of $\geq$1.2 mmol of BHBA/L in blood.

Within the last few years, excellent test characteristics were published for electronic hand-held devices, which were initially developed for the human medicine market. To our knowledge, so far, only devices distributed by Abbott Diabetes Care (Witney, UK) with differing labels were evaluated for BHBA testing in dairy cows (Jeppesen et al., 2006; Heuwieser et al., 2007; Iwersen et al., 2009; Voyvoda and Erdogan, 2010) and sheep (Panousis et al., 2012; Pichler et al., 2012).

The present study covers 3 objectives: (1) 2 new commercially available electronic hand-held devices [FreeStyle Precision (**FSP**), Abbott GmbH & Co. KG, Wiesbaden, Germany and GlucoMen LX Plus (**GLX**), A. Menarini GmbH, Vienna, Austria) were evaluated for detection of subclinical ketosis in dairy cows, (2) the effect of the type of blood sample (whole blood, EDTA-anticoagulated blood, serum, and plasma) was determined, and (3) the effects of the storage temperature of the device, the test strips, and the blood sample on the results of the devices were evaluated.

MATERIALS AND METHODS

The study was approved by the institutional ethics committee and the national authority according to §8ff of the Law for Animal Experiments [Tierversuchsgesetz (TVG); BMWF-68.205/0132-II/3b/2011]. Three experiments were conducted between January and December 2012. For all experiments, the 2 electronic hand-held devices (FSP and GLX) for determination of BHBA in whole blood were used. Each test system consists of an electronic hand-held meter and electrochemical test strips [FreeStyle Precision β-Ketone (Abbott Diabetes Care, Witney, UK) and GlucoMen LX β-Ketone Sensor (A. Menarini GmbH)]. In contrast to the already-evaluated Precision Xtra device (Abbott GmbH & Co. KG), calibration procedures of the meters before starting measurements are not necessary anymore. After inserting the test strips into the meter, a small amount of whole blood (1.5 μL for the FSP and 0.8 μL for the GLX device) is applied to the front edge of the sample application zone of the sensor, starting a chemical reaction within the test strips as follows: the BHBA in the blood sample is oxidized to AcAc in presence of the enzyme BHBA dehydrogenase, with the concomitant reduction of NAD^+ to NADH. The NADH is reoxidized to NAD^+ by a redox mediator. This chemical reaction releases electrons, generating a small current, which is directly proportional to the BHBA concentration in the sample. For both devices, test results are presented 10 s after application of the blood, presented as a digital value on the display of the meters. Referring to the manufacturer's manuals, the minimum operating temperature for both devices is +4°C.

Experiment 1

The Teaching and Research Farm (farm 1) of the University of Veterinary Medicine, Vienna (Austria), with approximately 80 Simmental cows, and a commercial dairy farm (farm 2) in Mecklenburg-Vorpommern, Germany, with approximately 600 Holstein-Friesian cows were used as study sites. Animals were housed in freestall barns with rubber mats on concrete floors in farm 1, and on slotted concrete floors in farm 2. Both farms were equipped with deep-bedded cubicles, filled either with straw (farm 1) or sawdust (farm 2). In both farms, a TMR was fed that was offered 8 times per day by an automatic feeding system (Trioliet-Mullos BV, Oldenzaal, the Netherlands) in farm 1, and twice per