Evaluation of 2 electronic handheld devices for diagnosis of ketonemia and glycemia in dairy goats

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ABSTRACT

The objective of this study was to evaluate the suitability of the electronic handheld devices FreeStyle Precision (FSP; Abbott Germany, Wiesbaden, Germany) and GlucoMen LX Plus (GML; A. Menarini GmbH, Vienna, Austria) for the measurement of β -hydroxybutyrate (BHBA) in whole blood in dairy goats. Additionally, glucose concentration was analyzed with the FSP device. For method comparison, the samples were also analyzed in the laboratory by standard methods, which served as the gold standard in our study. A further objective was to evaluate the agreement between samples obtained by minimal invasive venipuncture of an ear vein and measurements of whole blood samples from the jugular vein (vena jugularis). In total, 173 blood sample pairs collected from 28 goats were obtained from an ear vein and from the jugular vein. The Spearman correlation coefficients (r_{sp}) for BHBA concentrations determined with the FSP or GML and the gold standard were 0.95 and 0.85 for the ear vein and 0.98 and 0.88 for the jugular vein, respectively. Bland-Altman plots of differences showed a positive bias of 0.12 (ear vein) and 0.21 (jugular vein) when determination was performed with the FSP and a negative bias of 0.21 (ear vein) and 0.24 (jugular vein) when using the GML. For the FSP, applying the adjusted thresholds determined by ROC analysis of 0.9 (ear vein) and 1.0 mmol/L (jugular vein) allowed to distinguish between healthy goats and animals with hyperketonemia with sensitivities (Se) and specificities (Sp) for samples from the ear vein of 0.98 and 0.85, and from the jugular vein of 0.99 and 0.94, respectively. For the GML, adjusted thresholds were 0.5 mmol/L for the ear vein (Se = 0.94, Sp = 0.75) and 0.6 mmol/L for the jugular vein (Se = 0.88, Sp = 0.91). Repeated analyses of defined whole blood samples showed average inter- and intraassay coefficients of variation of 6.6

and 7.3% for FSP, and of 35.6 and 35.4% for GML, respectively. Test characteristics for determining glucose concentration with the FSP compared with the gold standard were poor (ear vein: r_{sp} = 0.41; jugular vein: r_{sp} = 0.51), with low validity to distinguish between hypo- and normoglycemia (Se = 0.71, Sp = 0.66). The present study showed good test characteristics for the FSP and moderate for the GML device for determining BHBA concentrations in whole blood. Additionally the results demonstrate the suitability of testing BHBA concentration in a blood drop obtained from an ear vein. Based on the results of this study, the FSP device is not suitable to differentiate normo- from hypoglycemia in goats.

Key words: goat, pregnancy toxemia, ketosis, handheld meter

INTRODUCTION

Disorders in the carbohydrate and fat metabolism are very common and can affect most species. In dairy goats, clinical ketosis manifests predominantly in early lactation (Stelletta et al., 2008). Does carrying multiple fetuses, undernourished dams, or animals in a very good body condition are predisposed to clinical ketosis (Rook, 2000; Brozos et al., 2011). Clinical cases of ketosis usually occur sporadically and unpredictably and can affect up to 2% of the herd (Rook, 2000). Mortality rates of the dams can reach up to 80% in the case of a problem within a herd (Andrews, 1997; Rook, 2000). Early detection of subclinical ketosis followed by an efficacious treatment should help to minimize economic losses (Rook, 2000; Sargison, 2007; Brozos et al., 2011).

An increased concentration of BHBA and low or physiological blood glucose concentrations are diagnostic hallmarks for ketosis (Andrews, 1997; Rook, 2000). To our knowledge, no validated thresholds to define hyperketonemia (HKET) in goats, showing negative effects on animal health or animal performance, have been reported yet. Hence, thresholds based on clinical experience in ewes as reported in the literature were used as decision criterion for HKET in the current study.

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Physiological BHBA concentrations in ewes are defined as serum concentrations <0.8 mmol/L (Rook, 2000). Concentrations of BHBA ≥ 0.8 mmol/L are indicative for maladaptation of the energy metabolism leading to impaired energy balance (Navarre et al., 2012). Animals with serum BHBA concentrations between 0.8 to 1.6 mmol/L are in a negative energy balance and thus at greater risk to develop clinical ketosis (Navarre et al., 2012). Animals with BHBA concentrations >1.6 mmol/L are in severe negative energy balance and, in combination with clinical symptoms, suffering from clinical ketosis (Rook, 2000). A study by Ismail et al. (2008) showed that pregnant does with subclinical ketosis had significantly lower glucose concentrations compared with nonketotic does. Whereas, in dairy cows, dipsticks can be used for detecting ketone bodies excreted in urine or milk (Carrier et al., 2004; Krogh et al., 2011), in small ruminants these semiquantitative tests are not as accurate as blood tests (Brozos et al., 2011). Electronic hand-held devices showed excellent test characteristics for measurement of BHBA and to some extent for glucose in whole blood in humans (Byrne et al., 2000; Noyes et al., 2007), cows (Voyvoda and Erdogan, 2010; Iwersen et al., 2013), and sheep (Panousis et al., 2012; Pichler et al., 2014). Recently, Doré et al. (2013) found an excellent correlation ($r = 0.98$; $P < 0.01$) between BHBA concentrations determined in whole blood of goats with the handheld meter Precision Xtra (Abbott Diabetes Care, Saint-Laurent, Canada) and serum BHBA concentrations analyzed at a laboratory.

The objective of the present study was to evaluate 2 electronic handheld devices, FreeStyle Precision (FSP; Abbott Germany, Wiesbaden, Germany) and GlucoMen LX Plus (GML; A. Menarini GmbH, Vienna, Austria), for on-site diagnosis of hyperketonemia in dairy goats. Additionally, the FSP was tested for measuring glucose concentrations in blood. A further objective was to evaluate the agreement between the results of BHBA and glucose concentrations in a single blood drop obtained from an ear vein and concentrations in whole blood from the jugular vein.

MATERIALS AND METHODS

Animals

The study was conducted on 28 White German milk goats (a composite of local white breeds and Saanen) commonly kept in Austria and Germany. The animals were owned by the Agricultural Research and Education Center Raumberg-Gumpenstein (Styria, Austria) and housed in 2 groups of 13 and 15 animals on deep litter. The goats had access to pasture during spring

and summer, and were fed twice per day with 0.6 kg of hay and 0.6 kg of concentrates per meal (18% soybean meal, 82% rolled barley grain). Before parturition, the does were kept separated in single boxes but with contact to the herd. After kidding, the does were regrouped. The goats were milked twice daily in a side-by-side milking parlor. The average milk yield of the herd was 924 kg per lactation with an average milk fat and protein content of 2.62 and 2.77%, respectively. All does within 5 wk before and up to 9 wk after parturition were enrolled in the study and checked visually for any health disorders before sampling. The study was approved by the Austrian Federal State Styria (FA10A-78Gu-15/2011-2) according to the Austrian Law for Animal Experiments.

Sampling Procedures and Measurement of BHBA and Glucose Concentrations

The present study was part of a longitudinal survey in dairy goats, where sampling was performed once a week starting 5 wk before to 6 wk after kidding, followed by an additional blood sample on wk 9 postpartum. Only a subset of these samples, collected in the kidding season between January to May 2012, were used to evaluate the handheld devices. Hence, individual animals were tested between 4 to 8 times within the study period. The metabolite BHBA was determined with the handheld devices FSP and GML in blood obtained by minimal invasive venipuncture of an ear vein and in whole blood from the jugular vein. Additionally, the glucose concentration was determined with the FSP at both sampling sites. In contrast to the already evaluated Precision Xtra device (Doré et al., 2013), the FSP and GML operate without prior calibration procedures.

For sampling procedures, the animal and animal's head were fixed by one person while a second person performed the venipuncture of an ear vein. For this, a well-visible vein was chosen and punctured with a cannula (Erosa Injektionskanüle, 1.2×50 mm, Rose GmbH, Trier, Germany), as described previously in ewes by Pichler et al. (2014). Performing this technique, a drop of whole blood of at least $0.60 \mu\text{L}$ was obtained and immediately analyzed with both handheld meters. Measurement procedures were repeated immediately in a blood sample obtained from the jugular vein, using a blood-collection tube system (Vacuette, Greiner Bio-One GmbH, Kremsmünster, Austria), serum tubes (Vacuette, Z-Serum Clot Activator, 9 mL, Greiner Bio-One GmbH), and a 20-gauge needle (Vacuette 0.9×38 mm, Greiner Bio-One GmbH). Measurement of BHBA was performed with the FSP and GML using FreeStyle Precision β -ketone strips (Abbott Diabetes Care, Wiltney, UK) and GlucoMen LX β -ketone sensor strips (A.

Menarini GmbH), respectively. Glucose was measured only with the FSP using the FreeStyle Precision glucose strips (Abbott Diabetes Care, Witney, UK) because the glucose test strips for the GML were not commercially available during most of the time of the study period. After blood sampling from the jugular vein, the sensor of the test strips were dipped directly onto the surface of the blood in the tubes for determining the BHBA and glucose concentration. After blood application on the test strips, BHBA concentrations were presented at the display of the FSP and GML after 10 s; glucose concentration was displayed at the FSP after 5 s.

For method comparison, the samples obtained from the jugular vein were also analyzed on a conventional large-scale analyzer for clinical chemistry at the laboratory of the Central Clinical Pathology Unit (CCPU), University of Veterinary Medicine Vienna (Vetmeduni Vienna, Austria). After approximately 2 h of clotting at ambient temperature (between 4 and 10°C), samples were centrifuged at $2,200 \times g$ for 10 min at room temperature (Eppendorf Centrifuge 5804, Eppendorf AG, Hamburg, Germany) and serum was separated and stored in 2 aliquots (2 mL, Micro tube, Sarstedt, Nümbrecht, Germany) at -25°C until analysis at the CCPU. Serum was analyzed with an automated wet chemistry analyzer Cobas 6000/c501 (Roche Diagnostics GmbH, Vienna, Austria). The BHBA concentration was determined by a colorimetric enzymatic test (Ranbut, D-3-hydroxybutyrate; Randox Laboratories Ltd., Crumlin, UK). This method is based on the oxidation of D-3-hydroxybutyrate to acetoacetate by the enzyme 3-hydroxybutyrate-dehydrogenase. The oxidation is coupled to an NAD indicator system. The induced color change is proportional to the BHBA concentration.

The serum glucose concentration was determined by an enzymatic colorimetric hexokinase method. Hexokinase catalyzes the phosphorylation of glucose to glucose-6-phosphate and adenosine-diphosphate by ATP. This reaction is coupled to an NADP color indicator system. The concentrations of BHBA and glucose determined in serum at the CCPU were defined as the gold standard in our study. The coefficients of variation were calculated for the wet chemistry methods used at the laboratory.

Evaluation of Precision

An additional aim of the current study was to evaluate the precision of BHBA measurements in whole blood with the 2 electronic handheld meters. For this, 3 blood samples with different BHBA concentrations (based on FSP measurements) of 0.4 (representing a physiological concentration: <0.8 mmol/L), 1.3 (representing moderate HKET: 0.8 to 1.6 mmol/L), and

1.6 mmol/L (representing severe HKET: >1.6 mmol/L) were analyzed with 10 different FSP and GML meters to calculate their specific interassay CV. To calculate the intraassay CV, each of the 3 blood samples was analyzed 10 times using a single FSP and GML device.

Statistical Analysis

Data were analyzed using the software packages PASW Statistics (version 17, IBM, Ehningen, Germany), MedCalc (version 12.7.7, MedCalc Software, Ostend, Belgium), and BiAS (version 9.07, Epsilon-Verlag, Darmstadt, Germany). The level of significance for all statistical tests was set at $P < 0.05$. The associations between the gold standard concentrations (serum BHBA and glucose) and the concentrations determined with the handheld devices in the blood drop of an ear vein and in whole blood from the jugular vein were calculated as Spearman correlation coefficients.

The agreement of the results between the handheld meters and the laboratory results were evaluated by Bland-Altman plots (Bland and Altman, 1986). The corresponding means, standard deviations, and binomial 95% confidence interval (CI_{95}) were calculated.

Based on serum BHBA concentrations determined at the CCPU, samples were classified as physiologic (<0.8 mmol/L), moderate HKET (0.8 to 1.6 mmol/L), and severe HKET (>1.6 mmol/L). According to the results of glucose determination at the CCPU, samples were classified as hypoglycemic (≤ 49 mg/dL), normoglycemic (50 to 63 mg/dL), and hyperglycemic (>63 mg/dL; Schwendenwein, 2009). Based on these classifications and thresholds, sensitivity (**Se**), specificity (**Sp**), and the Youden index (**J**) were calculated. The J, reflecting the number of all correctly identified outcomes, is calculated as $\text{Se} + \text{Sp} - 1$ (Youden, 1950).

Receiver operating characteristic (**ROC**) analyses were performed for adjusting the thresholds for the handheld devices (based on the laboratory results) to identify moderate and severe HKET, as well as to identify hypo- and hyperglycemia. The resulting area under the ROC curve (**AUC**) demonstrates the quality of the thresholds based on Se and Sp (Swets, 1988). Inter- and intraassay CV were determined to analyze the precision of the measurements of the 2 handheld meters. The CV was calculated as SD divided by the mean times 100. A one-way ANOVA (with Bonferroni post-hoc test) was assessed to evaluate differences between the glucose concentrations within the 3 BHBA classes.

RESULTS

A total of 173 pairs of blood samples (ear vein, jugular vein) were collected from 28 goats, with 154

sample pairs (89%) obtained from lactating does and 19 (11%) from nonlactating does in the last trimester of gestation. None of the animals showed any clinical sign of ketosis. All samples were analyzed for the concentration of BHBA with the FSP, GML, and at the CCPU. In 1 sample, the glucose concentration analyzed at the CCPU was below the minimum analytical detection limit. This sample was excluded from statistical analyses; hence, 172 pairs of samples were included in the analyses regarding glucose concentrations. The CV of the wet chemistry methods used at the CCPU were 3.6% for BHBA and 1.9% for glucose measurements.

Concentration of BHBA

The median BHBA concentration of all samples determined at the CCPU was 0.82 mmol/L, with an interquartile range of 0.51 mmol/L. Based on the laboratory results, 49% ($n = 85$) of the samples were classified as physiological, 45% ($n = 77$) as moderate hyperketonemic, and 6% ($n = 11$) as severe hyperketonemic. All samples with a serum BHBA concentration >0.8 mmol/L were obtained from lactating does. The BHBA and glucose concentrations, stratified by sampling time, are presented as boxplots in Figure 1. Samples collected antepartum up to wk 5 postpartum showed contrary alterations in BHBA and glucose concentrations. Approximately 27% (24 of 88) of the samples classified as HKET (BHBA ≥ 0.8 mmol/L) were additionally classified as hypoglycemic (glucose ≤ 49 mg/dL).

The Spearman correlation coefficient (r_{sp}) for the laboratory BHBA results and the concentration measured with the FSP was 0.95 (ear vein) and 0.98 (jugular vein), respectively (both $P < 0.01$). The r_{sp} for the laboratory BHBA concentration and the results determined with the GML were 0.85 (ear vein) and 0.88 (jugular vein), respectively (both $P < 0.01$). The r_{sp} for the BHBA concentration from whole blood of an ear vein and from the jugular vein determined with the FSP and GML were 0.94 and 0.85, respectively (both $P < 0.01$).

Figures 2 and 3 show Bland-Altman plots for differences between the gold standard (CCPU) and BHBA values determined with the FSP and GML. For the FSP, mean differences between CCPU and samples obtained from an ear vein and from the jugular vein were -0.12 ($CI_{95} = -0.48$ to 0.23) and -0.21 mmol/L ($CI_{95} = -0.59$ to 0.18). For the GML, mean differences between the gold standard and samples from an ear vein and from the jugular vein were 0.21 ($CI_{95} = -0.26$ to 0.68) and 0.24 mmol/L ($CI_{95} = -0.10$ to 0.58).

Based on the previous defined thresholds for moderate and severe HKET, ROC analyses were performed to

Figure 1. Box plots presenting the BHBA (a; $n = 173$) and glucose (b; $n = 172$) concentrations determined at the laboratory antepartum (a.p.) and postpartum (p.p.). The heavy black line inside each box marks the median (50th percentile); lower and upper hinges mark the 25th and 75th percentiles, respectively. Whiskers end at the smallest and largest statistical values that are not outliers; outliers and extreme values are designated by an open circle (○).

identify best thresholds for both handheld meters. The adjusted thresholds for the FSP and GML are presented in Table 1. To distinguish between healthy animals (BHBA <0.8 mmol/L) and does with moderate HKET (BHBA between 0.8 and 1.6 mmol/L), the optimized cutoff value for samples obtained from an ear vein with the FSP was 0.9 mmol/L (Se = 0.98; Sp = 0.85). For using the GML to distinguish between physiological and moderately high BHBA concentrations in blood from an ear vein, an optimized threshold of 0.5 mmol/L was calculated (Se = 0.94; Sp = 0.75). Table 1 presents

Figure 2. Bland-Altman plot of differences for the FreeStyle Precision handheld device (FSP; Abbott Diabetes Care Inc., Abbott Park, IL) to evaluate the BHBA concentration of the whole blood from an ear vein (a) and from the vena jugularis (v. jug.; b). The solid line in the middle shows the mean; the upper and lower solid lines present the mean $\pm$ SD.

also the results for the jugular vein and additional test characteristics (AUC, J).

Concentration of Glucose

The median of the glucose concentration determined at the CCPU was 45.0 mg/dL, with an interquartile range of 11.8 mg/dL. Overall, 75.0% ($n = 129$) of the samples showed serum glucose concentrations ≤ 49 mg/dL (hypoglycemic), whereas 23.8% ($n = 41$) were within the physiological range (50 to 63 mg/dL) and 2 samples (1.2%) were found with concentrations

Figure 3. Bland-Altman plot of differences for the GlucoMen LX Plus handheld device (GML; A. Menarini GmbH, Vienna, Austria) to evaluate the BHBA concentration of the whole blood from an ear vein (a) and from the vena jugularis (v. jug.; b). The solid line in the middle shows the mean; the upper and lower solid lines present the mean $\pm$ SD.

>63 mg/dL (hyperglycemic). Glucose concentrations in samples classified as normoketotic (<0.8 mmol/L) were 3.87 mg/dL greater than in samples classified as moderate HKET ($P = 0.02$) and 8.87 mg/dL greater compared with samples classified as severe HKET ($P < 0.01$).

The Spearman correlation coefficient for glucose concentrations measured at the CCPU and in a blood drop from an ear vein with the FSP was 0.41 ($P < 0.01$); the correlation coefficient for the gold standard and whole blood from the jugular vein was $r_{sp} = 0.51$ ($P < 0.01$). The correlation coefficient for glucose concentrations

Table 1. Receiver operating characteristic (ROC) analysis for the handheld meters Freestyle Precision (FSP; Abbott Diabetes Care Inc., Abbott Park, IL) and GlucoMen LX Plus (GML; A. Menarini GmbH, Vienna, Austria) of whole blood from an ear vein and from the vena jugularis in dairy goats (samples, $n = 173$)

Threshold BHBA concentration ¹	Handheld device	Optimized cutoff ² (mmol/L)	Se ³ (CI ₉₅) ⁴	Sp ⁵ (CI ₉₅)	AUC ⁶ (CI ₉₅)	J ⁷
0.8 mmol/L						
Ear vein	FSP	0.9	0.98 (0.92–0.99)	0.85 (0.75–0.92)	0.96 (0.92–0.99)	0.82
	GML	0.5	0.94 (0.87–0.98)	0.75 (0.64–0.84)	0.92 (0.87–0.96)	0.69
Vena jugularis	FSP	1.0	0.99 (0.94–1.00)	0.94 (0.87–0.98)	0.99 (0.97–1.00)	0.94
	GML	0.6	0.88 (0.80–0.94)	0.91 (0.82–0.96)	0.93 (0.90–0.97)	0.78
1.6 mmol/L						
Ear vein	FSP	1.9	1.00 (0.72–1.00)	0.99 (0.96–1.00)	0.99 (0.98–1.00)	0.99
	GML	1.2	1.00 (0.72–1.00)	0.97 (0.93–0.99)	0.99 (0.96–0.99)	0.97
Vena jugularis	FSP	2.0	1.00 (0.72–1.00)	0.99 (0.96–1.00)	0.99 (0.98–1.00)	0.99
	GML	1.3	0.91 (0.59–1.00)	0.99 (0.97–1.00)	0.99 (0.96–0.99)	0.90

¹Serum BHBA concentration determined at the laboratory.²Optimized cutoff value for the FreeStyle Precision and GlucoMen LX Plus devices.³Sensitivity = percentage of ketotic does that tested positive.⁴CI₉₅ = 95% confidence interval.⁵Specificity = percentage of nonketotic does that tested negative.⁶AUC = area under the ROC curve.⁷Youden index = (Se + Sp – 1); deviations are caused by rounding.

determined with the FSP from whole blood of an ear vein and the jugular vein was $r_{sp} = 0.74$ ($P < 0.01$). The Bland-Altman plots in Figure 4 illustrate the mean differences between the laboratory results and the FSP in samples obtained from an ear vein (-6.3 mg/dL; CI₉₅ = -26.5 to 13.9) and from the jugular vein (-10.9 mg/dL; CI₉₅ = -28.1 to 6.2).

To calculate optimized thresholds for determining glucose concentration with the FSP, ROC analyses were performed. For identifying hypo- and hyperglycemic goats with the FSP using a blood drop from an ear vein, a threshold of 51.5 (Se = 0.71 , CI₉₅ = 0.62 to 0.78 ; Sp = 0.66 , CI₉₅ = 0.53 to 0.82) and 66.0 mg/dL (Se = 1.00 , CI₉₅ = 0.16 to 1.00 ; Sp = 0.95 , CI₉₅ = 0.95 to 0.99) were determined. The calculated AUC of the thresholds of 51.5 and 66.0 mg/dL were 0.65 (CI₉₅ = 0.60 to 0.75) and 0.98 (CI₉₅ = 0.97 to 1.00), with a J of 0.36 and 0.95 . Corresponding thresholds for whole blood from the jugular vein were 58.5 (Se = 0.81 , CI₉₅ = 0.73 to 0.87 ; Sp = 0.63 , CI₉₅ = 0.49 to 0.79) and 80.0 mg/dL (Se = 1.00 , CI₉₅ = 0.16 to 1.00 ; Sp = 1.00 , CI₉₅ = 0.98 to 1.00). The AUC of the thresholds of 58.5 and 80.0 mg/dL were calculated as 0.74 (CI₉₅ = 0.68 to 0.82) and 1.00 (CI₉₅ = 0.98 to 1.00), with J of 0.44 and 1.00 .

Inter- and Intraassay CV

The average inter- and intraassay CV for determining BHBA concentrations with the FSP were 6.6 and 7.3% , respectively. Corresponding CV for the GML were 35.6 (interassay) and 35.4% (intraassay). It should

be mentioned that the evaluation of the intra- and interassay agreement had to be repeated, because 80% of the measurements with the GML device resulted in error messages.

DISCUSSION

Our study presents a systematic evaluation of the 2 electronic handheld devices FreeStyle Precision and GlucoMen LX Plus for determining BHBA concentrations in goats. The mechanism of subclinical ketosis in goats is not well studied (Andrews, 1997; Sargison, 2007). A therapeutic intervention at an early and moderate stage of the disease, however, is considered as more effective than a treatment of severe HKET (Andrews, 1997; Sargison, 2007). The current study was not designed to demonstrate the prevalence of HKET or the treatment efficacy, but to evaluate the handheld meters for an accurate diagnosis of HKET. The discussion focuses on subclinical HKET with BHBA concentrations >0.8 mmol/L, because the number of samples with BHBA concentrations >1.6 mmol/L was low in our study and none of the does showed any clinical sign of ketosis. Thresholds used in our study were derived from ewes, because no validated BHBA concentrations were available in literature as indicative for HKET in dairy goats. Hence, further studies should focus on evaluating ewe-specific thresholds for HKET, considering their reproductive status. Additionally, further studies may evaluate the accuracy of determined BHBA concentrations from goats suffering from clinical ketosis and the effect of an early intervention on treatment efficacy.

Figure 4. Bland-Altman plot of differences for the FreeStyle Precision handheld device (FSP; Abbott Diabetes Care Inc., Abbott Park, IL) to evaluate the glucose concentration of the whole blood from an ear vein (a) and from the vena jugularis (v. jug.; b). The solid line in the middle shows the mean; the upper and lower solid lines present the mean $\pm$ SD.

As the energy requirements of does considerably increase with the onset of lactation, most does develop a mild or lactational ketosis. Goats in early lactation lose excessive BW if the feed intake or quality is not adequate to meet the energy requirements. Hence, animals are in a negative energy balance (Matthews, 2009). This could be a major reason for the high percentage (51%, $n = 88$) of samples classified as HKET (BHBA ≥ 0.8 mmol/L) in the present study. Supporting this hypothesis, all of these samples were obtained from animals after kidding.

A strong positive correlation was found for the FSP between the concentration of BHBA analyzed from the

whole blood of the jugular vein and the gold standard ($r_{sp} = 0.98$); consistent with the study of Doré et al. (2013; $r = 0.98$). The BHBA determination with the GML presented lower corresponding correlation coefficients of $r_{sp} = 0.88$. A study on dairy cows by Iwersen et al. (2013) reported similar correlation coefficients for whole blood and serum BHBA concentrations analyzed with the FSP ($r_{sp} = 0.94$) and the GML ($r_{sp} = 0.80$) as in the present study.

To our knowledge, ours is the first study in dairy goats that compares the results from 2 different sampling sites (ear vein and jugular vein) for the evaluation of determination of BHBA and glucose concentrations. Using minimally invasive blood sampling techniques, as already established in small animal medicine (Zeugswetter et al., 2010), might also be beneficial in livestock for monitoring of HKET. These sampling sites were chosen because they are commonly used in veterinary practice (jugular vein) or could be easily achieved (ear vein). The correlation coefficient between the BHBA concentration in whole blood of an ear vein and the jugular vein determined with the FSP were high ($r_{sp} = 0.94$; $P < 0.01$), but lower when measurement was conducted with the GML ($r_{sp} = 0.85$; $P < 0.01$).

The correlation between BHBA concentration in serum and in whole blood from an ear vein measured with the FSP was excellent ($r_{sp} = 0.95$; $P < 0.01$), indicating that the on-site test is suitable for use in veterinary practice; again, the GML presented a lower corresponding correlation coefficient of $r_{sp} = 0.85$. The minimal invasive venipuncture of an ear vein was tested in a previous study to identify HKET in ewes and showed similar results for the Precision Xceed ($r_{sp} = 0.94$; Pichler et al., 2014). The venipuncture of goats with small ears might be more challenging and should be tested in further studies.

Bland-Altman plots revealed a positive or negative bias for the FSP and GML compared with the gold standard. The plots of the FSP showed a wider variation with increasing BHBA concentrations. Hence, adjusted clinical decision limits were calculated by ROC analysis. This procedure improved test characteristics for both devices. Test characteristics were slightly better for the FSP than for the GML. This is similar to findings by Iwersen et al. (2013), who also found differences in test characteristics between the 2 devices in dairy cows.

A potential weakness of our study is the number of enrolled animals and the composition of the sample set, which is based on repeated measurements of individual animals. The 11 samples classified as severe HKET, were obtained from 7 animals tested once and from 2 animals tested twice. Calculating the specific Se and Sp for each handheld device by sampling site and time

revealed an overlapping corresponding CI_{95} in 95% (61 of 64) of the compared parameters. Hence, independence was assumed for the corresponding sampling times. However, further studies based on a larger and more diverse sample population should be performed to confirm the evaluated thresholds.

The inter- and intraassay coefficients of variation for the FSP were both below 10%, which is similar to the recently published data from Doré et al. (2013), who found CV of 5.25 and 3.73% for a similar device. Coefficients of variation for the GML, however, were both greater than 35%. Reasons for these differences remain speculative and might be related not to the device but to the test strips. The test strip measurement error of the GML during determining the CV was very high (80%) and tests had to be repeated. Meanwhile BHBA test strip packages for the GML were modified by the manufacturer, because of their possible effect on the test results. It could be of interest if blood volumes applied to the GML test strips had an effect on the test results. In accordance with the "Guideline on bioanalytical method validation," published by the European Medicines Agency (EMA, 2011), low, medium, and high BHBA concentrations were chosen for the determination of the CV. Using more samples at different BHBA concentrations with less replications might be beneficial to dilute the effect of any matrix effect. Additionally, further research should be conducted to evaluate the inter- and intraassay CV of the glucose concentration.

In many cases, hyperketonemia is associated with decreased blood glucose concentrations (Andrews, 1997; Rook, 2000; Mavrogianni and Brozos, 2008). These findings are referred to as type I ketosis in dairy cows, when inhibited gluconeogenesis from body protein leads to low plasma glucose levels while the concentrations of ketones are high (Holtenius and Holtenius, 1996). In our study, 27% of the animals classified as HKET were additionally classified as hypoglycemic.

According to the threshold of ≤ 49 mg/dL for serum glucose concentrations, 75% of the serum samples were classified as hypoglycemic. The reason for this finding remains speculative. In serum samples, blood glucose concentrations decrease by ongoing glycolysis, which is dependent on storage temperature (Rehak and Chiang, 1988; Morris et al., 2002). In the present study, clotting was allowed for a maximum of 2 h and at temperatures between 4 to 10°C. Hence, under these storage and handling conditions, the effects of glycolysis might be negligible, as reported in the literature (Zhang et al., 1998; Morris et al., 2002). The low concentrations of glucose might also be explained by using serum clot tubes and analyzing serum not plasma. Morris et al. (2002) found higher glucose concentrations in ovine

plasma collected with sodium heparinized tubes compared with serum. A study by Kuwa et al. (2001) determined a difference of the glucose concentration between mean values of venous whole blood and venous plasma of 11.3%. Although, in the present study, venous serum was analyzed at the CCPU, it cannot be excluded that collecting systems and substrates have influenced the proportion of samples classified as hypoglycemic.

The correlations between the glucose concentrations determined at the CCPU and in the whole blood of an ear vein or the jugular vein with the FSP were very poor ($r_{sp} = 0.41$ and 0.51 ; both $P < 0.01$) and the Bland-Altman plots revealed that the FSP underestimated the glucose concentrations. Even the correlation coefficient for the concentrations determined with the FSP from whole blood of an ear vein and jugular vein was only $r_{sp} = 0.74$ ($P < 0.01$). Because of the low number of hyperglycemic samples, evaluated thresholds to define hyperglycemia using the FSP are not reliable. Hence, glucose concentrations determined with the FSP should be interpreted with care. Adjusted thresholds for identifying hypoglycemic samples did not result in satisfying test characteristics.

CONCLUSIONS

After adjusting the thresholds, the FSP device is suitable for analyzing BHBA concentration in goats from the whole blood of the jugular vein and from blood obtained by minimally invasive venipuncture of an ear vein. The GML device showed also good correlations with the gold standard, but the inter- and intraassay coefficients of variation were very high and need further studies for validation. The FSP showed poor test characteristics for determining glucose concentrations and is not suitable for an accurate diagnosis of hypoglycemia.

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